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November 1, 1980
volume 138, number 5

CUK-H01387-17-P024277

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American Journal of OBSTETRICS AND GYNECOLOGY

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Published by

THE C. V. MOSBY COMPANY
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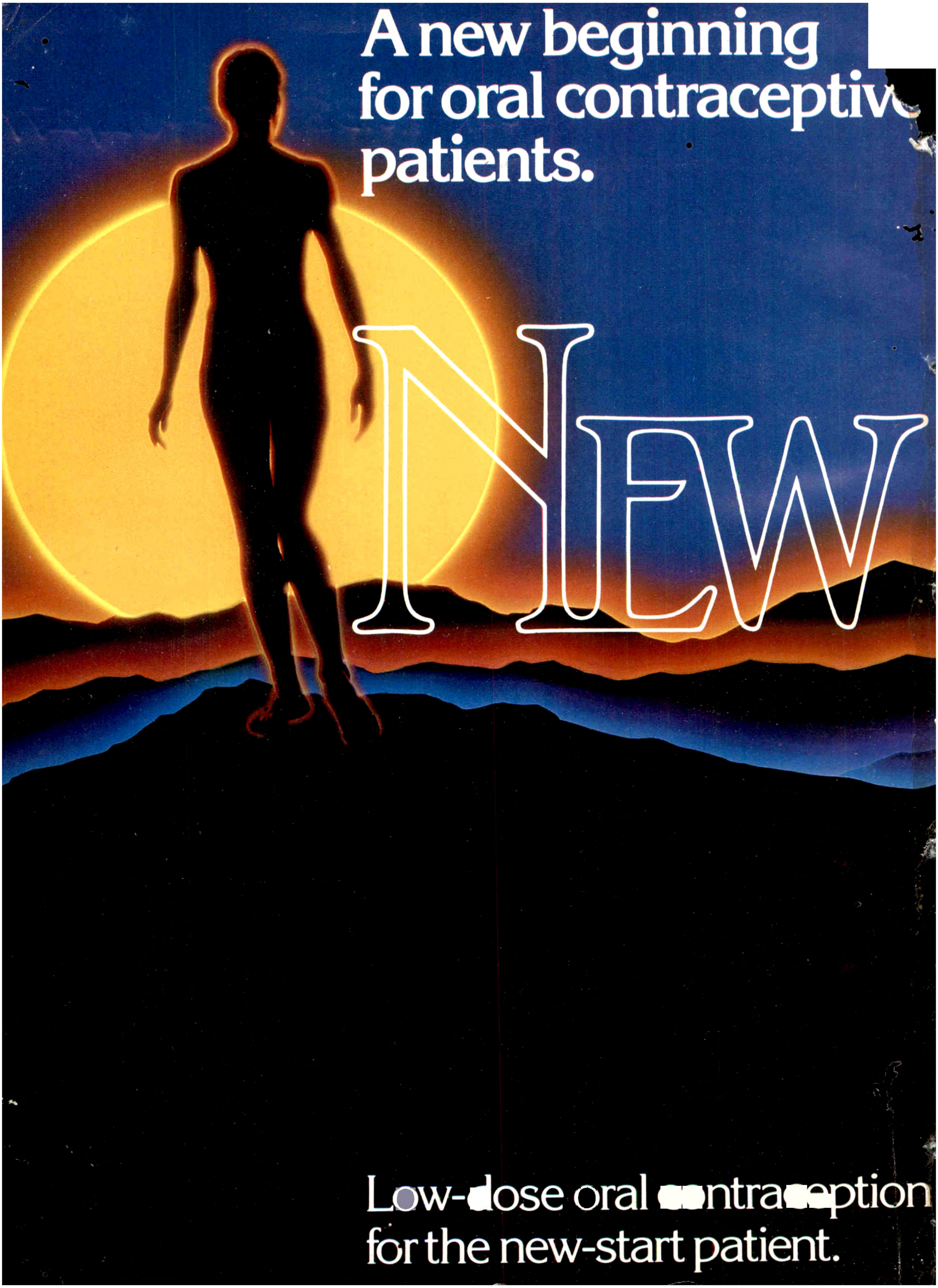
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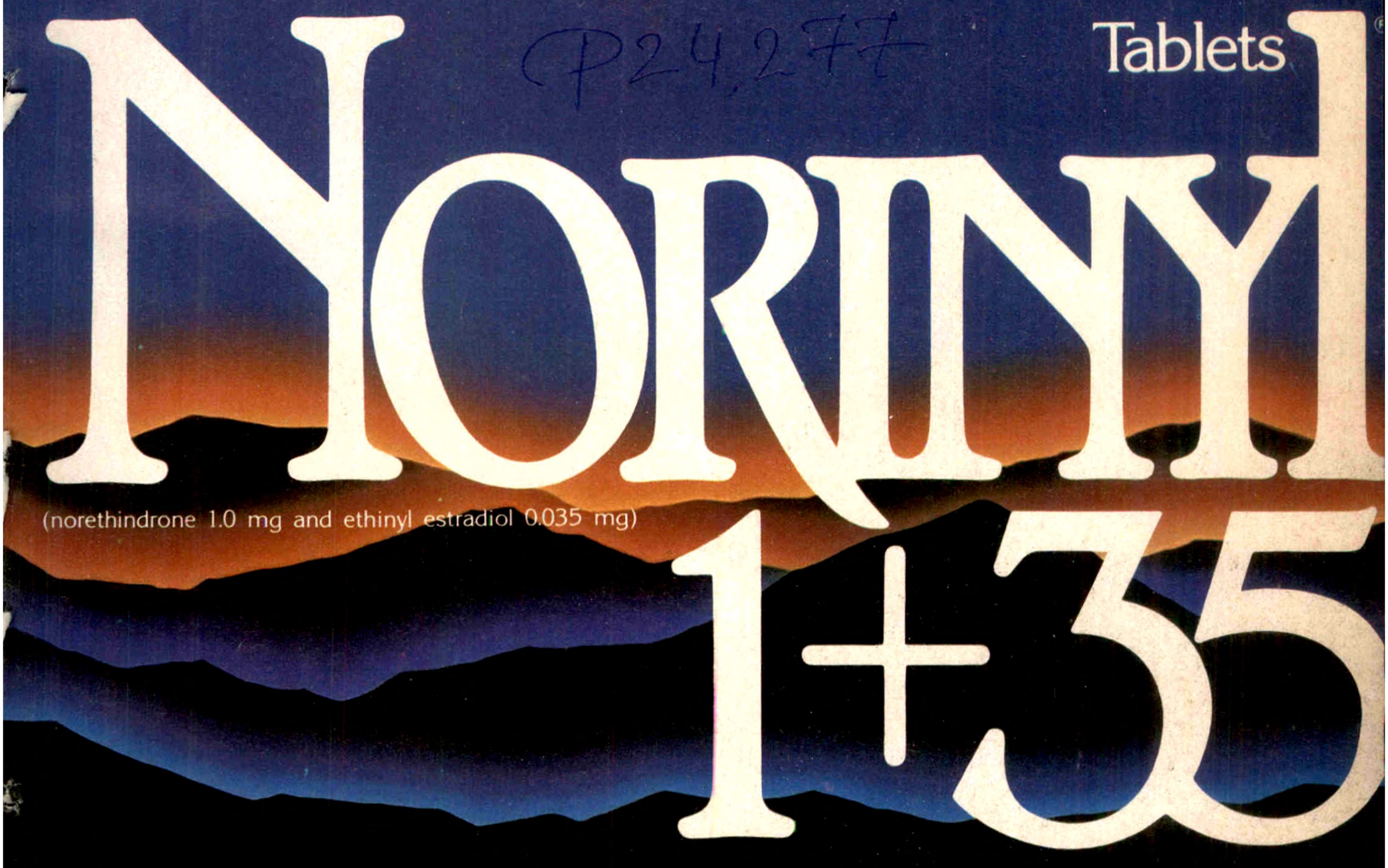

(norethindrone 1.0 mg and ethinyl estradiol 0.035 mg)

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*Note: serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see summary of Prescribing Information on following page.

†The overall pregnancy rate was 0.17 per hundred woman-years, in clinical trials with 940 patients completing 14,366 cycles of use.

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ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications O.C.s are indicated for the prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM FROM O.C.s.* Studies have shown a positive association between the dose of estrogens in O.C.s and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The O.C. product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of O.C.s should be started on preparations containing 0.05 mg or less of estrogen. **Contraindications** 1. Known or suspected pregnancy (see Warning No. 5). 2. Thrombophlebitis or thromboembolic disorders. 3. A past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. O.C.s should not be used by women who have or have had any of the following conditions: a. Cerebral vascular or coronary artery disease. b. Known or suspected carcinoma of the breast. c. Known or suspected estrogen dependent neoplasia. d. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen containing products.

> **WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from O.C. use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use O.C.s should be strongly advised not to smoke.**
>
> The use of O.C.s is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing O.C.s should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of O.C.s is well established. One British study demonstrated an increased relative risk for fatal venous thromboembolism; two British and three American studies demonstrated an increased relative risk for non-fatal venous thromboembolism. These studies estimate that users of O.C.s are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke, which had not been shown in prior British studies. In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the relative risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between O.C. use and stroke. Another British long-term, follow-up study had suggested such an association in 1974, but the number of cases was too small to estimate the risk. MYOCARDIAL INFARCTION. An increased relative risk of myocardial infarction associated with the use of O.C.s has been reported, confirming a previously suspected association. One study conducted in the United Kingdom found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an O.C. user or not. O.C.s, however, were found to be an additional risk factor. In terms of relative risk, it has been estimated that O.C. users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. O.C. users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, and about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the number of cigarettes smoked is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. (The estimates are based on British vital statistics which show acute myocardial infarction death rates 2- to 3-times less than in the U.S. for women in these age groups; consequently, actual U.S. death rates could be higher.) The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in O.C.s. Preparations containing 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been supported by a study in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only O.C.s have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASE. A large British prospective study estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of O.C.s according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for O.C. users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). The risk is concentrated in older women, in those with a long duration of use, and in cigarette smokers, and may persist after discontinuation of O.C. use. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of O.C.s) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The findings of this analysis are shown in Table 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that of childbirth, with the exception of O.C.s in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates given would be about double; for "light" smokers (less than 15 cigarettes a day), about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The study also concluded that O.C. users who smoke, especially those over the age of 30, have a greater mortality risk than O.C. users who do not smoke.

Table 1. *The annual number of deaths associated with control of fertility and no control per 100,000 nonsterile women by regimen of control and age of women are:*

	15-19	20-24	25-29	30-34	35-39	40-44
No method	5.6	6.1	7.4	13.9	20.8	22.6
Abortion only	1.2	1.6	1.8	1.7	1.9	1.2
Pill only —nonsmokers	1.3	1.4	1.4	2.2	4.5	7.1
Pill only —smokers	1.5	1.6	1.6	10.8	13.4	58.9
IUDs only	0.9	1.0	1.2	1.4	2.0	1.9
Traditional contraception only	1.1	1.6	2.0	3.6	5.0	4.2
Traditional contraception and abortion	0.2	0.2	0.3	0.3	0.3	0.2

The risk of thromboembolic and thrombotic disease associated with O.C.s increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclampic toxemia and especially by cigarette smoking. Based on risk of death from circulatory disorders associated with the use of O.C.s:

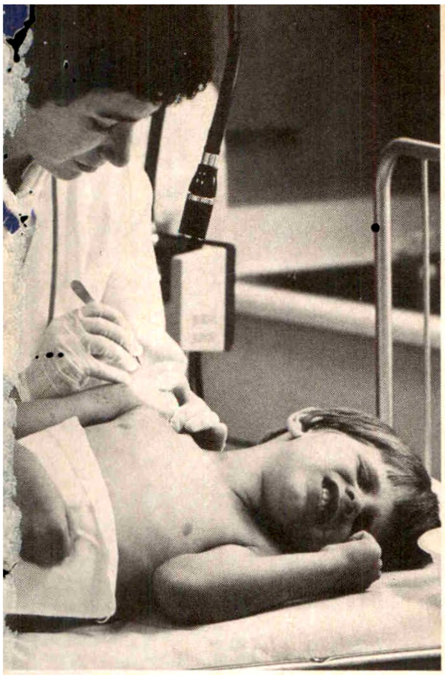
Table 2. *Smoking habits and other predisposing conditions—risk associated with use of O.C.s*

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (No predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A—Use associated with very high risk.
B—Use associated with high risk.
C—Use associated with moderate risk.
D—Use associated with low risk.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis) Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of post-surgery thromboembolic complications has been reported in O.C. users. If feasible, O.C.s should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. Data also suggest that the presence of varicose veins substantially increases the risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. *Ocular Lesions:* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of O.C.s. Discontinue O.C. medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of certain adenomas, benign or malignant, such as those of the breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently contained in O.C.s, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies have reported an increased relative risk (3.1 to 13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women who took estrogen replacement medication to relieve menopausal symptoms. One publication reported on the first 30 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on O.C.s. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time O.C.s were first given, polycystic ovaries) nearly all occurred in women who had used a sequential O.C. These products are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only O.C.s, although individual cases have been reported. Several studies have shown no increased risk of breast cancer to women taking O.C.s or estrogens. In one study, however, while no overall increased risk of breast cancer was noted in women treated with O.C.s, a greater risk was suggested for the subgroups of O.C. users with documented benign breast disease and for long-term (2-4 years) users. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. A reduced occurrence of benign breast tumors in users of O.C.s has been well documented. In a prospective study of women with cervical dysplasia, there was an increase in severity and of conversion to cancer *in situ* in O.C. users compared with nonusers. This became statistically significant after 3 to 4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to be predictive of progression after prolonged exposure. There have been other reports of microglandular hyperplasia of the cervix in users of O.C.s. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with O.C.s. Close clinical surveillance of all women taking O.C.s is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with O.C. use. This has been reported in short-term as well as long-term users of O.C.s. One study reported that use of O.C.s with high hormonal potency and age over 30 years may further increase a woman's risk of hepatocellular adenoma. Two studies relate risk with duration of use, the risk being much greater after 4 or more years of use. Although it is an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may clearly show a focal defect. Hepatic arteriography may be a useful procedure in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that O.C.s further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. Furthermore, a high percentage of women exposed to diethylstilbestrol (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported following use of sex hormones, including O.C.s, in pregnancy. In one case-control study it was estimated that there was a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (O.C.s, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including O.C.s, during early pregnancy occurred at a rate of 18.2 per 1,000 births, compared to 7.8 per 1,000 for children not so exposed *in utero*. These results are statistically significant. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing O.C.s. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping O.C.s is unknown. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if progestogen-only O.C.s are used) and further use of O.C.s should be withheld until pregnancy has been ruled out. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue O.C.s with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of

bladder disease in users of O.C.s or estrogens. In one study, an increase[illegible] risk appeared after 2 years of use and doubled after 4 or 5 years of use. [illegible] another study an increased risk was apparent between 6 and 12 months o[illegible] use. 7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on O.C.s For this reason, prediabetic and diabetic patients should be carefully observed while receiving O.C.s. An increase in triglycerides and total phospholipids has been observed in patients receiving O.C.s. The clinical significance of this finding is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with O.C. use. In some women, hypertension may occur within a few months of beginning O.C. use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in O.C. use[illegible] Women with a history of elevated blood pressure (hypertension), preexisting renal disease, a history of toxemia or elevated blood pressure during pregnancy, a familial tendency to hypertension or its consequences, or a history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevation of blood pressure when given O.C.s and, therefore, should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, the clinical implications of elevations should not be ignored and close surveillance is indicated, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after discontinuation of the O.C. [illegible] *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of O.C.s and evaluation of the cause. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses are frequent reasons for patients discontinuing O.C.s. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an O.C. with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of O.C.s. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased prevalence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing O.C.s in comparison with those discontinuing other methods of contraception. The impairment appears to be independent of the duration of use of the preparations. While the impairment diminishes with time, there is still an appreciable difference in the results in nulliparous women for the O.C. and non-O.C. groups 30 months after discontinuation of birth control. For parous women the difference is no longer apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only O.C. failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving O.C.s, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* O.C.s given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in O.C.s has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast fed child have not been determined. If feasible, the use of O.C.s should be deferred until the infant has been weaned. **Precautions** *GENERAL* 1. A complete medical and family history should be taken prior to the initiation of O.C.s. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, O.C.s should not be prescribed for longer than 1 year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking O.C.s should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. 4. O.C.s may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving O.C. therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. O.C. users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is unknown. 8. Serum folate levels may be depressed by O.C. therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping O.C.s, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of O.C. therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing O.C.s. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. *DRUG INTERACTIONS* O.C.s may be rendered less effective by virtue of drug interaction with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, and antimigraine preparations. O.C.'s may alter the effectiveness of other types of drugs, such as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., quanethidine), vitamins and hypoglycemic agents. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reations has been associated with the use of O.C.s (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving O.C.s and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening) change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors. The follwing adverse reactions have been reported in users of O.C.s, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function. **Information for the Patient** (See Patient Package Insert)



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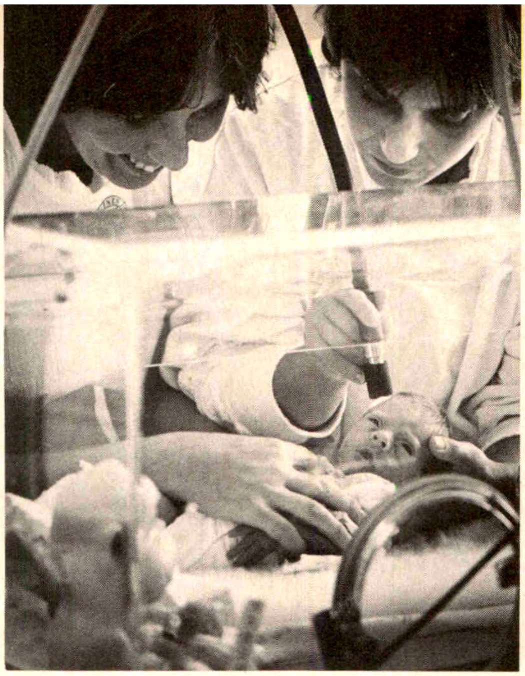

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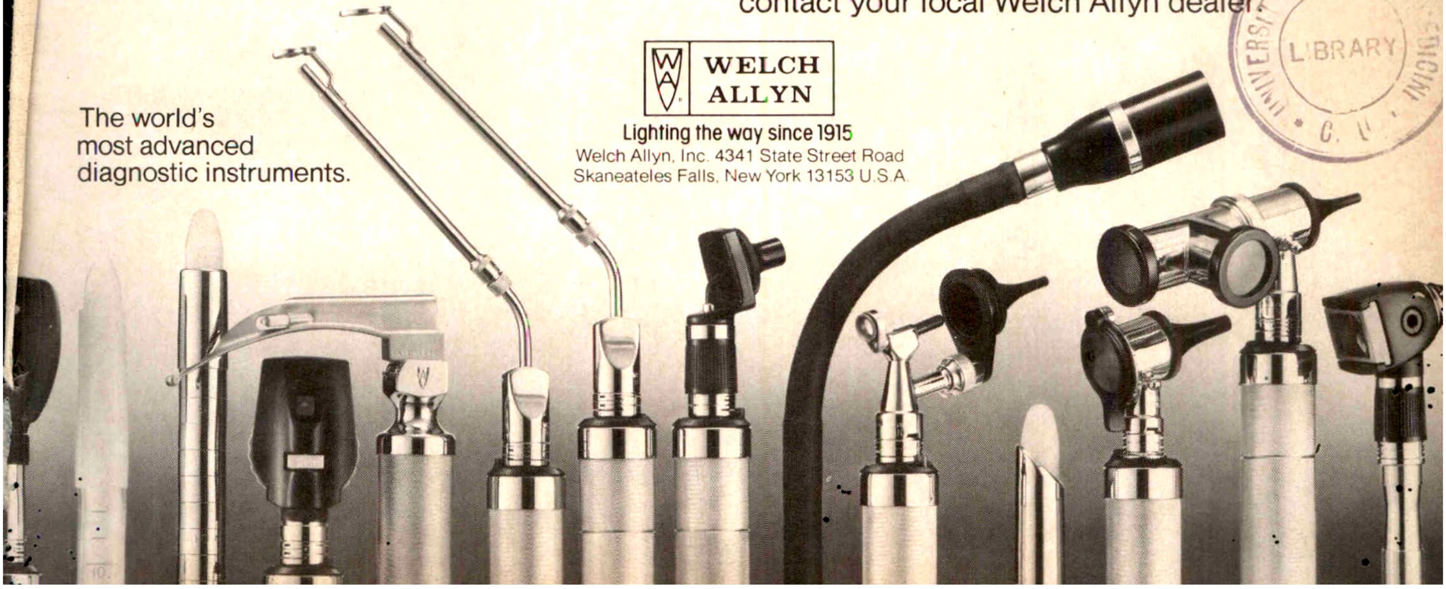

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DESCRIPTION: Each tablet contains aspirin (acetylsalicylic acid) 325 mg plus codeine phosphate in one of the following strengths: No. 2 — 15 mg, No. 3 — 30 mg, and No. 4 — 60 mg. (Warning — may be habit-forming.) CIII

CONTRAINDICATIONS: Hypersensitivity to aspirin or codeine.

WARNINGS:

Drug dependence: Empirin with Codeine can produce drug dependence of the morphine type and, therefore, has the potential for being abused. Psychic dependence, physical dependence, and tolerance may develop upon repeated administration of this drug and it should be prescribed and administered with the same degree of caution appropriate to the use of other oral, narcotic-containing medications. Like other narcotic-containing medications, the drug is subject to the Federal Controlled Substances Act.

Use in ambulatory patients: Empirin with Codeine may impair the mental and/or physical abilities required for the performance of potentially hazardous tasks such as driving a car or operating machinery. The patient using this drug should be cautioned accordingly.

Interaction with other central nervous system (CNS) depressants: Patients receiving other narcotic analgesics, general anesthetics, phenothiazines, other tranquilizers, sedative-hypnotics, or other CNS depressants (including alcohol) concomitantly with Empirin with Codeine may exhibit an additive CNS depression. When such combined therapy is contemplated, the dose of one or both agents should be reduced.

Use in pregnancy: Safe use in pregnancy has not been established relative to possible adverse effects on fetal development. Therefore, Empirin with Codeine should not be used in pregnant women unless, in the judgment of the physician, the potential benefits outweigh the possible hazards.

PRECAUTIONS:

Head injury and increased intracranial pressure: The respiratory depressant effects of narcotics and their capacity to elevate cerebrospinal fluid pressure may be markedly exaggerated in the presence of head injury, other intracranial lesions or a pre-existing increase in intracranial pressure. Furthermore, narcotics produce adverse reactions which may obscure the clinical course of patients with head injuries.

Acute abdominal conditions: The administration of Empirin with Codeine or other narcotics may obscure the diagnosis or clinical course in patients with acute abdominal conditions.

Allergic: Precautions should be taken in administering salicylates to persons with known allergies: patients with nasal polyps are more likely to be hypersensitive to aspirin.

Special risk patients: Empirin with Codeine should be given with caution to certain patients such as the elderly or debilitated, and those with severe impairment of hepatic or renal function, hypothyroidism, Addison's disease, prostatic hypertrophy or urethral stricture, peptic ulcer, or coagulation disorders.

ADVERSE REACTIONS: The most frequently observed adverse reactions to codeine include light-headedness, dizziness, sedation, nausea and vomiting. These effects seem to be more prominent in ambulatory than in nonambulatory patients and some of these adverse reactions may be alleviated if the patient lies down. Other adverse reactions include euphoria, dysphoria, constipation, and pruritus.

The most frequently observed reactions to aspirin include headache, vertigo, ringing in the ears, mental confusion, drowsiness, sweating, thirst, nausea, and vomiting. Occasional patients experience gastric irritation and bleeding with aspirin. Some patients are unable to take salicylates without developing nausea and vomiting. Hypersensitivity may be manifested by a skin rash or even an anaphylactic reaction. With these exceptions, most of the side effects occur after repeated administration of large doses.

DOSAGE AND ADMINISTRATION: Dosage should be adjusted according to the severity of the pain and the response of the patient. It may occasionally be necessary to exceed the usual dosage recommended below in cases of more severe pain or in those patients who have become tolerant to the analgesic effect of narcotics. Empirin with Codeine is given orally. The usual adult dose for Empirin with Codeine No. 2 and No. 3 is one or two tablets every four hours as required. The usual adult dose for Empirin with Codeine No. 4 is one tablet every four hours as required.

DRUG INTERACTIONS: The CNS depressant effects of Empirin with Codeine may be additive with that of other CNS depressants. See WARNINGS.

Wellcome

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Research Triangle Park
North Carolina 27709

American Journal of Obstetrics and Gynecology

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Vol. 138, No. 5, November 1, 1980. The American Journal of Obstetrics and Gynecology is published semimonthly by The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63141. POSTMASTER: Send address changes to above address.

		Foreign countries (surface mail)	*Foreign countries (airmail)**		
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Institutional†	$58.50	$86.50	$139.15	$187.15	$235.15
Individual‡	$39.50	$67.50	$120.15	$168.15	$216.15
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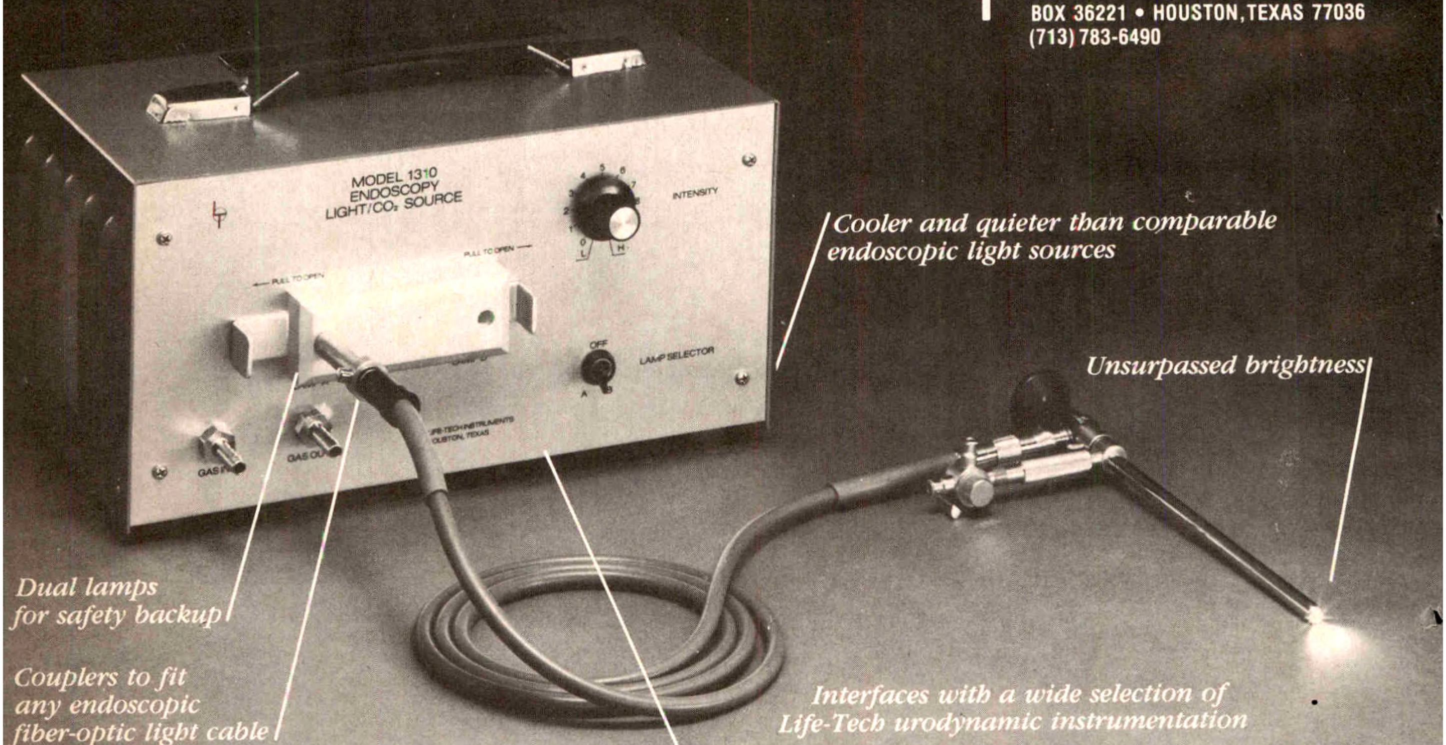

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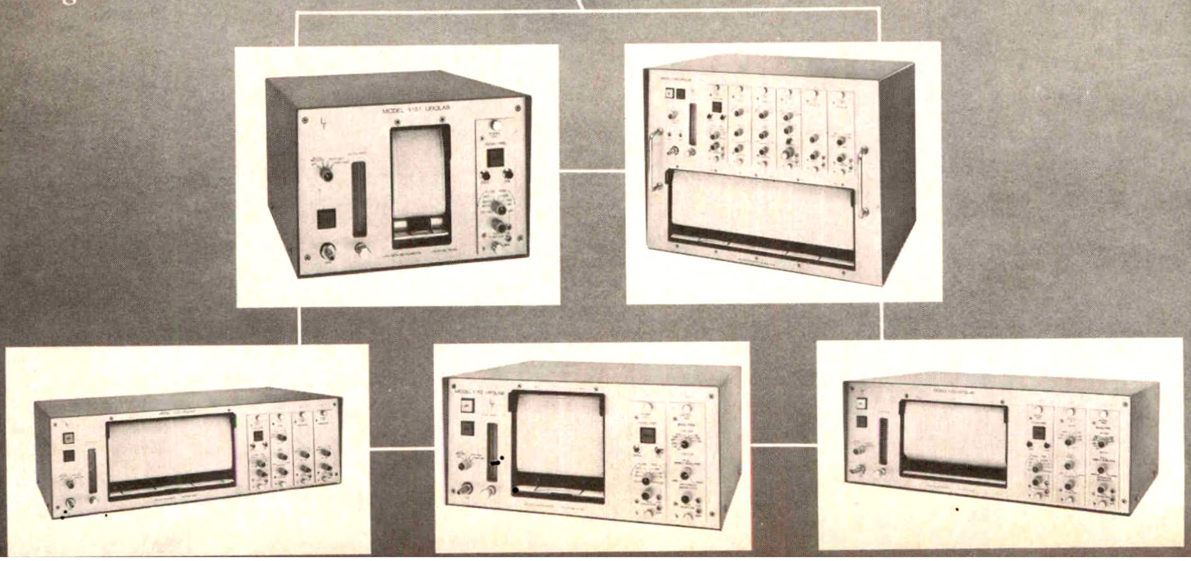

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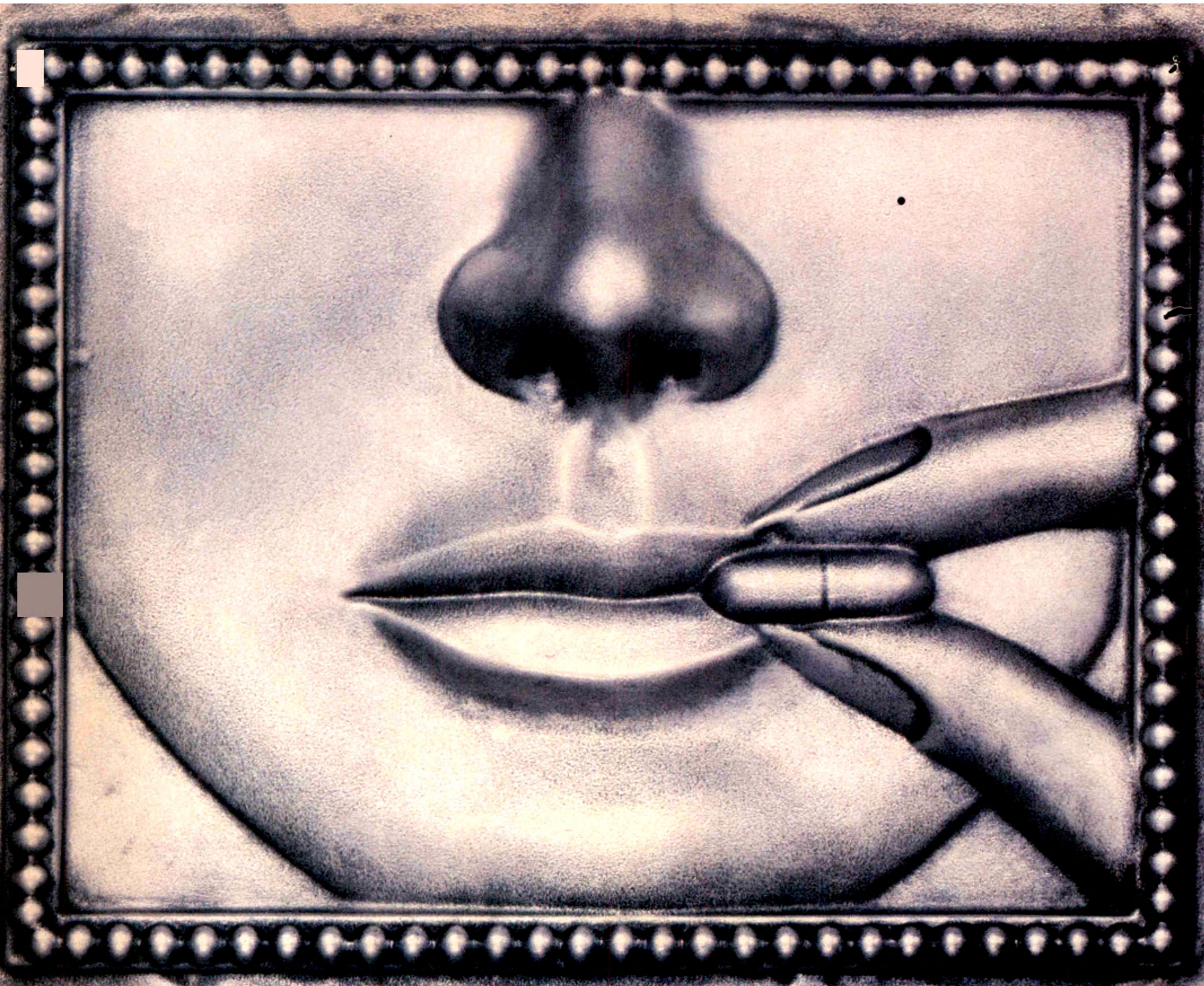

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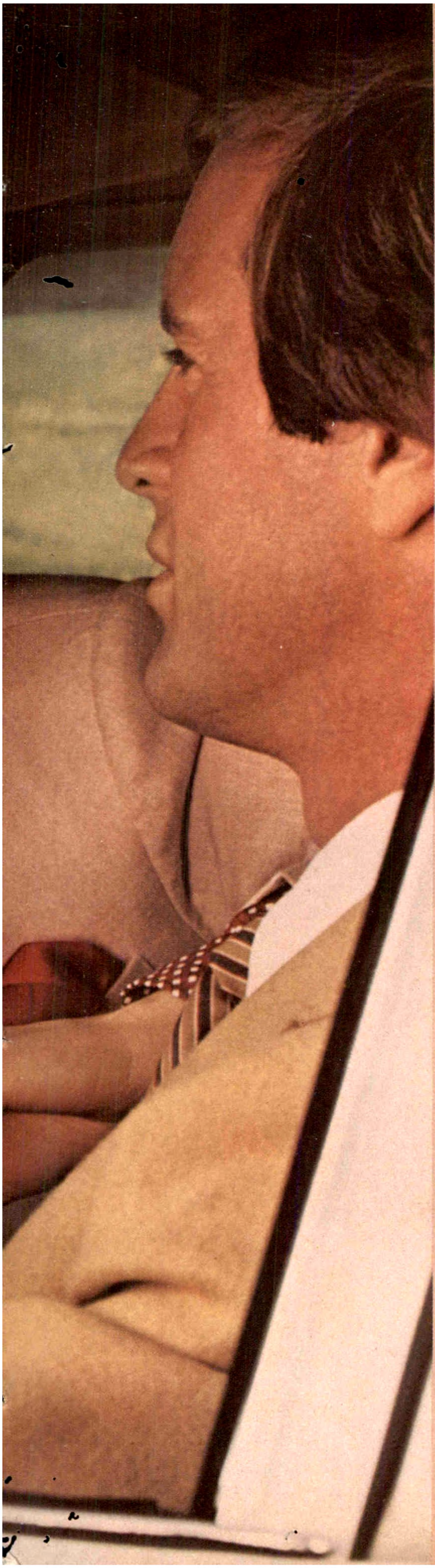

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LOESTRIN® 1.5/30 limits estrogen exposure and often reduces the frequency of estrogen-related side effects, because it contains just 30 mcg of ethinyl estradiol. LOESTRIN 1.5/30 combines this low estrogen dose with norethindrone acetate, a progestin that's been used alone for years in gynecologic therapy. Together, they offer more than 99% effective oral contraception.

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Brief Summary of Prescribing Information

LOESTRIN® 1.5/30

(1.5 mg norethindrone acetate and 30 mcg ethinyl estradiol tablets, USP) See section under Special Notes on Administration and How Supplied.

DESCRIPTION

Loestrin 1.5/30 Products are progestogen-estrogen combinations.

INDICATION AND USAGE

Loestrin 1.5/30 Products are indicated for the prevention of pregnancy in women who elect to use oral contraceptives.

In clinical trials with Loestrin 1.5/30 involving 17,139 therapy cycles, there was a pregnancy rate of 0.49 per 100 woman years.

Dose-related risk of thromboembolism from oral contraceptives: Studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. It is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance.

CONTRAINDICATIONS

1. Thrombophlebitis or thromboembolic disorders
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders
3. Cerebral vascular or coronary artery disease
4. Known or suspected carcinoma of the breast
5. Known or suspected estrogen-dependent neoplasia
6. Undiagnosed abnormal genital bleeding
7. Known or suspected pregnancy (See No. 5 under WARNINGS.)

WARNINGS

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
>
> The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, and hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well-established. Studies have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic.

Cerebrovascular disorders: In a collaborative study in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 4.0 to 9.5 times greater.

Myocardial infarction: An increased risk of myocardial infarction associated with oral contraceptives has been reported confirming a previously suspected association. These studies found that the greater the number of underlying risk factors (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) for coronary artery disease, the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor.

It has been estimated that users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are smokers have about a fivefold increased risk of fatal infarction compared to users who do not smoke, but about a tenfold to twelvefold increased risk compared to nonusers who do not smoke. The amount of smoking is also an important factor.

Risk of dose: In an analysis of data, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; however, the quantity of estrogen may not be the sole factor involved.

Estimate of excess mortality from circulatory diseases: The risk of diseases of the circulatory system is concentrated in older women, in those with a long duration of use, and in cigarette smokers.

A study of available data from a variety of sources concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke.

The risk of thromboembolic and thrombotic diseases associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders. Should any occur or be suspected, the drug should be discontinued immediately.

A fourfold to sixfold increased risk of postsurgery thromboembolic complications has been reported in users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

2. *Ocular lesions:* Neuro-ocular lesions, such as optic neuritis or retinal thrombosis, have been associated with the use of oral contraceptives. Discontinue the oral contraceptive if there is unexplained sudden or gradual, partial, or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions.

3. *Carcinoma:* Long-term continuous administration of estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver.

In humans, an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women has been reported. However, there is no evidence suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives.

Studies found no evidence of increase in breast cancer in women taking oral contraceptives; however, an excess risk in users with documented benign breast disease was reported.

There is no confirmed evidence of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of users is, nevertheless, essential. In cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer, or who have breast nodules, fibrocystic disease, or abnormal mammograms, should be monitored with particular care.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with oral contraceptives. Because hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage, they should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock.

A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Usage in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* During early pregnancy, female sex hormones may seriously damage the offspring.

An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of oral contraceptives in pregnancy.

There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives.

Pregnancy should be ruled out before continuing an oral contraceptive in any patient who has missed two consecutive menstrual periods. If the patient has not adhered to the schedule, the possibility of pregnancy should be considered at the time of the first missed period, and oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed.

Women who discontinue oral contraceptives with the intent of becoming pregnant should use an alternate form of contraception for a period of time before attempting to conceive.

Administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives.

7. *Carbohydrate and Lipid Metabolic Effects:* Because decreased glucose tolerance has been observed in a significant percentage of patients, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives.

An increase in triglycerides and total phospholipids has been observed.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving oral contraceptives. The prevalence in users increases with longer exposure. Age is also strongly correlated with development of hypertension. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure.

9. *Headache:* Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, nonfunctional causes should be borne in mind. In undiagnosed abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy.

Women with a past history of oligomenorrhea or secondary amenorrhea, or young women without regular cycles should be advised that they may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast Feeding:* Oral contraceptives may interfere with lactation. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs.

PRECAUTIONS

1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another examination.
2. Preexisting uterine leiomyomata may increase in size.
3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.
4. Oral contraceptives may cause fluid retention and should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated.
5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice. If jaundice develops, the medication should be discontinued.
6. Steroid hormones may be poorly metabolized and should be administered with caution in patients with impaired liver function.
7. Users may have disturbances in normal tryptophan metabolism, which may result in a relative pyridoxine deficiency.
8. Serum folate levels may be depressed.
9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted.
10. Certain endocrine and liver function tests and blood components may be affected.

(a) Increased sulfobromophthalein retention. (b) Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. (c) Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone. (d) Decreased pregnanediol excretion. (e) Reduced response to metyrapone test.

Drug interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin and an association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and ampicillin.

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with oral contraceptives.

Thrombophlebitis; Pulmonary embolism; Coronary thrombosis; Cerebral thrombosis; Cerebral hemorrhage; Hypertension; Gallbladder disease; Benign hepatomas; Congenital anomalies.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed. Mesenteric thrombosis, Neuro-ocular lesions, eg, retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms; breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma; breast changes; change in weight; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately post partum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature; intolerance to contact lenses.

The following adverse reactions have been reported and the association has been neither confirmed nor refuted.

Premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis; porphyria.

Special Notes on Administration

Loestrin 21 1.5/30 (1.5 mg norethindrone acetate and 30 mcg ethinyl estradiol tablets, USP) — Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after discontinuing medication.

Loestrin Fe 1.5/30 — Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after the brown ferrous fumarate tablets have been started.

Because of the relatively low estrogenic content, Loestrin 1.5/30 is not a good cyclic regulator. There are patients whose inherent hormone balance will require larger amounts of estrogen than that contained in Loestrin 1.5/30 to achieve cyclic regularity. These patients experience altered bleeding patterns, which do not conform to treatment schedules.

The physician should be alert to the fact that the irregular bleeding patterns could mask bleeding from organic cause, and appropriate diagnostic measures should be taken if the bleeding persists or continues after changing to a higher estrogen-content product.

After several months, bleeding may be reduced to a virtual absence; reduced flow may be a result of medication and not indicative of pregnancy.

HOW SUPPLIED

Loestrin Fe 1.5/30 is available in compacts each containing 21 green tablets and 7 brown tablets. Each green tablet contains 1.5 mg of norethindrone acetate and 30 mcg of ethinyl estradiol. Each brown tablet contains 75 mg of ferrous fumarate. Available in packages of five compacts and packages of five refills.

Loestrin 21 1.5/30 is available in compacts each containing 21 tablets. Each green tablet contains 1.5 mg of norethindrone acetate and 30 mcg of ethinyl estradiol. Available in packages of five compacts and packages of five refills.

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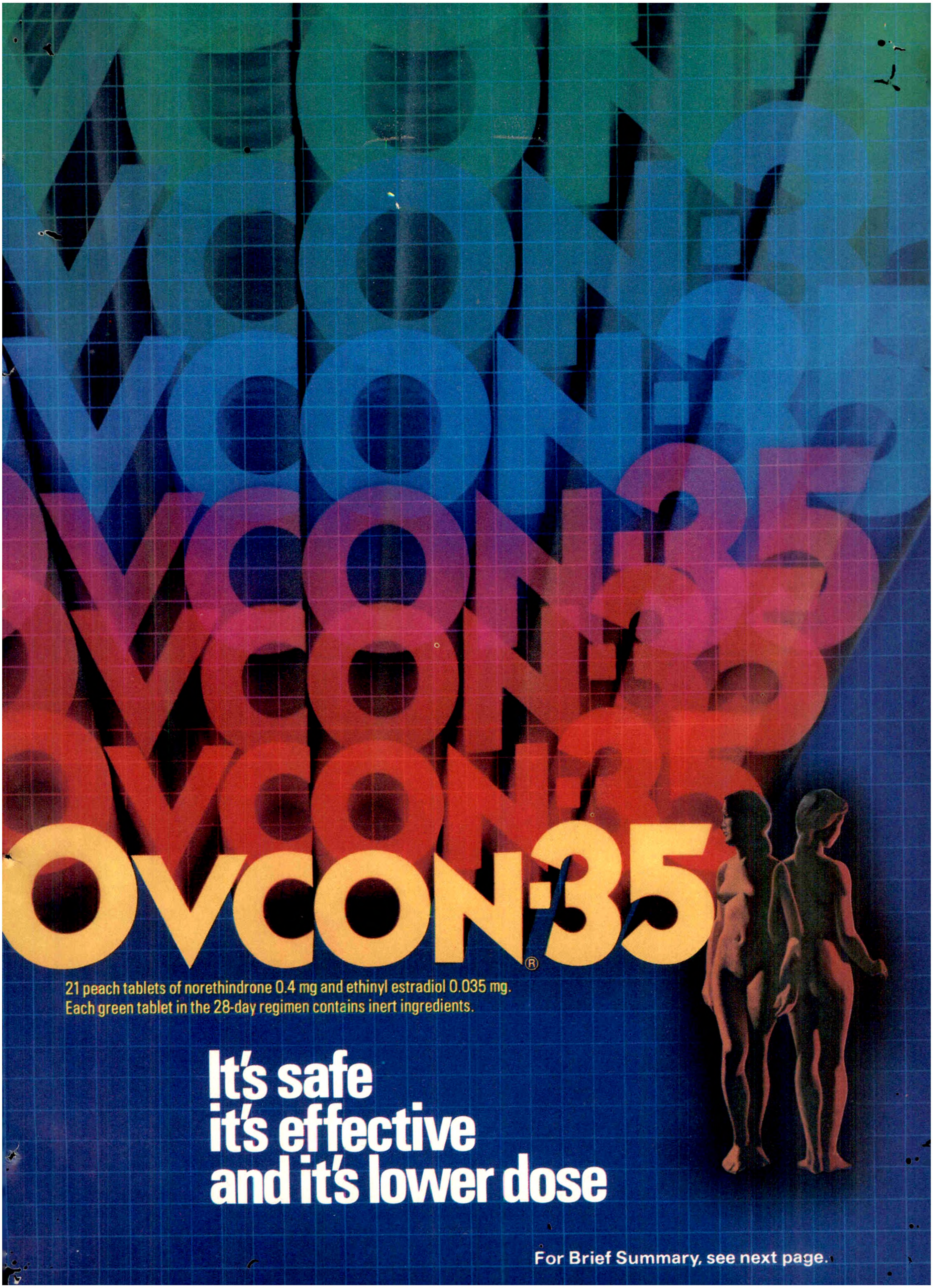

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Each green tablet in the 28-day regimen contains inert ingredients.

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21 peach tablets of norethindrone 0.4 mg and ethinyl estradiol 0.035 mg. Each green tablet in the 28-day regimen contains inert ingredients.

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg. or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy.

Warnings:

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.**

1) *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg. or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg. of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-35, 5/100,000; ages 35-44, 33/100,000; ages 45-49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) *Ocular Lesions.* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) *Carcinoma.* Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) *Hepatic Adenoma.* Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) *Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.* Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) *Gall Bladder Disease.* Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) *Carbohydrate and Lipid Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) *Elevated Blood Pressure.* An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) *Headaches.* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) *Ectopic Pregnancy.* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) *Breast Feeding.* A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG, free T_4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium and ampicillin.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (Other reactions, as a general rule, are seen much less frequently or only occasionally.); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, intolerance to contact lenses, change in corneal curvature (steepening), cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Mead Johnson
PHARMACEUTICAL DIVISION

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American Journal of Obstetrics and Gynecology

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Tables should be typed on separate sheets of paper, not in the text, with one table to a page. They should be numbered in sequence and each must be referred to at an appropriate point in the text. Captions of the tables should be brief, yet indicate clearly the purpose or content of each table. Rows and columns in the table should precisely define the nature of the data in each. Abbreviations in tables should be used as little as possible and if abbreviations are used they should be explained in a footnote to the table.

An abstract of 50 to 150 words, to be published as an introduction, should accompany each manuscript and should be typed on a separate sheet of paper.

A footnote should be included which gives the name and complete mailing address of the person to whom reprint requests and correspondence should be sent.

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Announcements. Announcements of meetings must be received by Dr. Brewer at least 2½ months prior to the time of the meeting. Such announcements should concern major meetings and other significant activities. Announcements of symposia or seminars for which fees are charged are not published in the scientific pages of the JOURNAL but may be submitted for paid advertisements, if desired.

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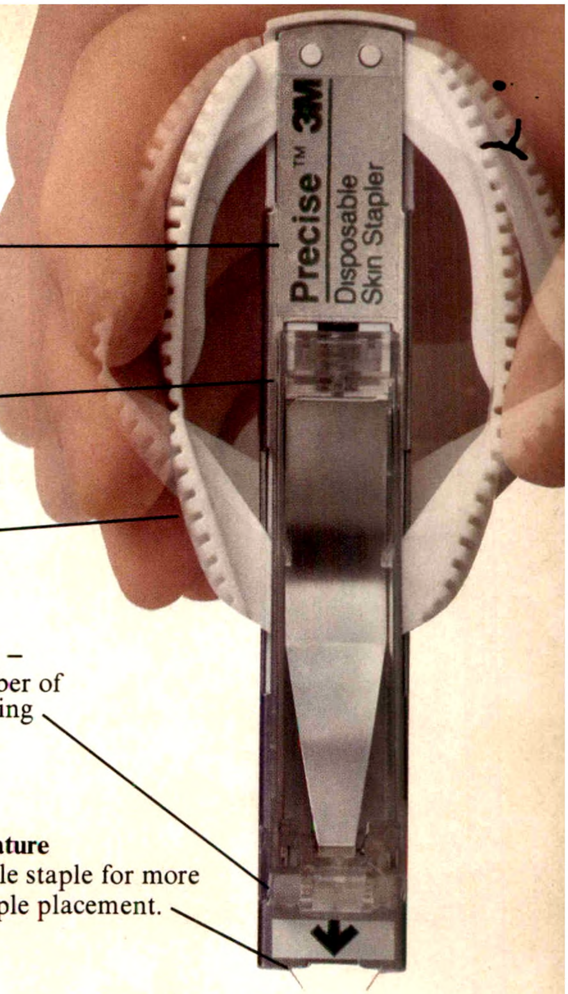

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†Serious as well as minor side effects have been reported with the use of oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. The physician should be fully aware of the Complete Prescribing Information for this product.

complete prescribing information certain text, tables, and references. The physician should be thoroughly familiar with the complete prescribing information and patient information before prescribing the product.

INDICATION: CONTRACEPTION. The pregnancy rate in women using conventional combination oral contraceptives (containing 3[illegible]mcg or more of ethinyl estradiol or 50 mcg or more of mestranol) is generally reported as less than one pregnancy per 100 woman-years of use. Slightly higher rates (somewhat more than one pregnancy per 100 woman-years of use) are reported for some combination products containing 35 mcg or less of ethinyl estradiol, and rates on the order of three pregnancies per 100 woman-years are reported for the progestogen-only oral contraceptives. Table 1 gives ranges of [illegible]ancy rates reported in the literature for other means of contraception. The efficacy of these means of contraception (except the IUD) depends upon the degree of adherence to the method.

Table 1: Pregnancies Per 100 Women-Years. IUD, less than 1-6; Diaphragm with spermicidal product (creams or jellies), 2-20; Condom, 3-36; Aerosol foams, 2-29; Jellies and creams, 4-36; Periodic abstinence (rhythm) all types, less than 1-47; 1. Calendar method, 14-47; 2. Temperature method, 1-20; 3. Temperature method—intercourse only in postovulatory phase, less than 1-7; 4. Mucus method, 1-25; No contraception, 60-80. **DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES:** Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen. **CONTRAINDICATIONS:** Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Known or suspected pregnancy (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.
The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION: An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10-to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of oral contraceptives) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The findings of this analysis are shown in Figure 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. Based on the data currently available, the following chart gives a gross estimate of the risk of death from circulatory disorders associated with the use of oral contraceptives:

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF ORAL CONTRACEPTIVES

Age	Below 30	30-39	40+	
Heavy smokers	C	B	A	A—Use associated with very high risk.
Light smokers	D	C	B	B—Use associated with high risk.
Nonsmokers (no predisposing conditions)	D	C,D	C	C—Use associated with moderate risk.
Nonsmokers (other predisposing conditions)	C	C,B	B,A	D—Use associated with low risk.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception.

*Trademark

No method	5.5	5.2	7.1	14.0	19.3	21.9
Abortion only	2.3	2.5	2.5	5.2	9.8	6.6
Pill only-nonsmokers	1.3	1.4	1.4	2.2	4.5	3.1
Pill only-smokers	1.5	1.6	1.6	10.8	13.4	58.9
IUDs only	1.1	1.2	1.2	1.4	1.6	1.4
Traditional contraception only	1.1	1.4	1.9	3.7	4.7	4.0
Traditional contraception and abortion	0.3	0.4	0.4	0.8	1.4	0.8

4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, and tetracycline. **CARCINOGENESIS. PREGNANCY. NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. **ACUTE OVERDOSE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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ORTHO PHARMACEUTICAL CORPORATION
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Revised 6/80

American Journal of Obstetrics and Gynecology

volume 138 *number* 5 NOVEMBER 1, 1980

P24,277

CLINICAL OPINION

This section reports opinion on the handling of clinical situations, i.e., the clinical diagnosis and management of certain disease entities. Papers should range from eight to twenty typed pages, including illustrations, tables, and figures which clarify the author's management. References are limited to sixteen citations. Mail to Frederick P. Zuspan, M.D., Editor.

Percentile ranks of sonar fetal abdominal circumference measurements

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We present the percentile ranks of sonar fetal abdominal circumference (AC) measurements from 18 to 41 weeks' gestation. The ACs are derived from both longitudinal and cross-sectional ultrasonic studies of 200 low-risk pregnant women. The reproducibility of sonar AC falls within 2% of the mean value; this variation permits antenatal distinction of the fetus with a small AC (less than twenty-fifth percentile) from one with an average (twenty-fifth to seventy-fifth percentile) or large (greater than eightieth percentile) reading. The fetal AC measurements add another dimension to the interpretation of cephalic growth, particularly in identifying macrosomic fetuses as well as those who are either asymmetrically or symmetrically undergrown. Additionally fetal AC measurements are useful as adjuncts to the diagnosis of hydrocephalus by quantitating the difference between cephalic and body size. In the presence of fetal ascites the AC also can be used to assess the severity and progression of the abnormality. (AM. J. OBSTET. GYNECOL. 138:475, 1980.)

IT IS POSSIBLE to evaluate fetal growth by ultrasonically derived measurements of biparietal diameter (BPD), chest diameter (CD), and abdominal circumference (AC).[1-4, 6] Serial sonar BPD values, obtained at appropriate intervals in pregnancy, are particularly useful in defining gestational age and in assigning fetuses a cephalic percentile growth bracket.[7, 8]

Fetal AC, however, is more useful than either BPD or CD for estimation of fetal weight (Table I), especially when the predicted weight is ≤2,500 grams.[5, 6] Further, in the presence of intrauterine growth retar-

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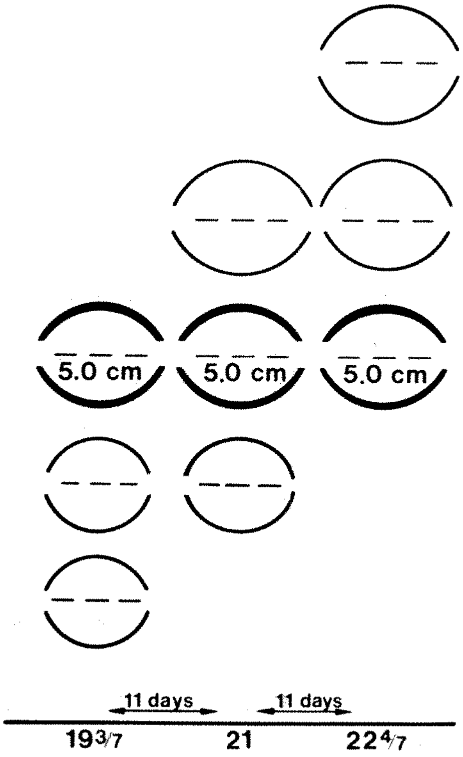

Fig. 1. A biologic variation in the length of the BPD exists in relation to each gestational week. Thus, a fetus with a BPD of 5.0 cm may be either less or more mature than the mean gestational age by 11 days. *Note:* In relation to the mean gestational age, the fetus with the large BPD is less mature and the fetus with the small BPD is more mature.

Table I. Fetal ultrasonic BPD and AC measurements versus fetal weight

No. of studies	*Parameter*	*Imaging modality*	*Approximate range in weight (gm ± 2 SD)*
2[1,2]	BPD	B	650-750
1[3]	BPD and thorax	B	580
1[4]	Skull and thoracic area	B	430
1[5]	BPD and AC	B	*Related to actual weight* 212 at 1,000 gm 424 at 2,000 gm 636 at 3,000 gm 848 at 4,000 gm
1[6]	AC	B	*Related to actual weight:* 160 at 1,000 gm 290 at 2,000 gm 450 at 3,000 gm 590 at 4,000 gm

Table II. A comparison of sonar and actual fetal AC measurements (in centimeters)

*Sonar AC**	*Actual AC†*
36.0	34.0
36.1	34.5
35.5	33.0
34.6	32.5
33.3	32.0
31.7	30.5
34.8	32.5
34.9	32.0
21.8	23.0
20.8	21.0
31.0	32.0

*Sonar AC measurements were obtained on the evening of admission for repeat cesarean section.

†Actual AC measurements were obtained by a centimeter tape from fetuses within 24 hours after delivery by repeat cesarean section. AC readings shown are at the xyphoid level. AC values obtained within 1 cm of the xyphoid were within 2% of the xyphoid AC.

dation (IUGR), the AC is likely to be small because growth of the liver is stunted[9] and the subcutaneous tissue in the area of the upper abdomen is diminished, both of which influence the abdominal girth.

While BPD growth standards have already been reported,[7] the range of AC values corresponding to each gestational week is unknown. In this report the percentile ranks of sonar fetal ACs from the eighteenth to forty-first week of pregnancy are defined. Additionally the reproducibility and accuracy of AC measurements are assessed.

Patients, material, and methods

Serial ultrasonic BPD and AC fetal measurements were prospectively obtained in 200 women with low-risk pregnancies and of different racial origins but of similar socioeconomic status; the data were derived between 18 and 42 weeks' gestation. The population was divided into two groups.

In the first group of 92 pregnant women, 40 were black, 29 were Caucasian, and 23 were Spanish. In all of these women gestational age was defined from the menstrual dates after the following criteria were fulfilled: (1) The last menstrual period (LMP) was reported with certainty and menstrual cycles were known to have been regular prior to pregnancy; (2) spontaneous delivery occurred at term (±2 weeks of the menstrual expected date of delivery or EDD); and (3) the neonate was found to be at term (38 to 42 weeks of pregnancy) by pediatric assessment.[10]

In the second group of 108 pregnant women, gestational age was defined after the following criteria were fulfilled: (1) The first fetal sonar BPD was derived in the midtrimester of pregnancy and correlated with a mean gestational age of 18 to 26 weeks. (2) A second fetal BPD was obtained in the interval of 31 to 33 weeks' gestation and a growth-adjusted sonar age (GASA) was used to correct the length of pregnancy in fetuses with large or small BPDs.[7] Specifically, when

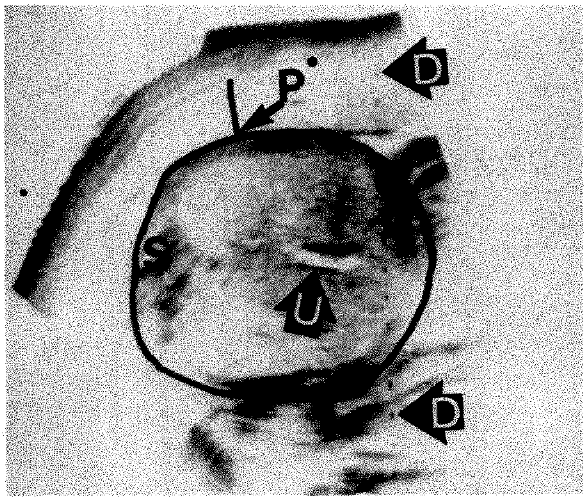

Fig. 2. The AC is in a circle. *P* = Point at which measurement of AC is started; *S* = spine; *U* = umbilicus; *D* = dots representing 1 cm marker display.

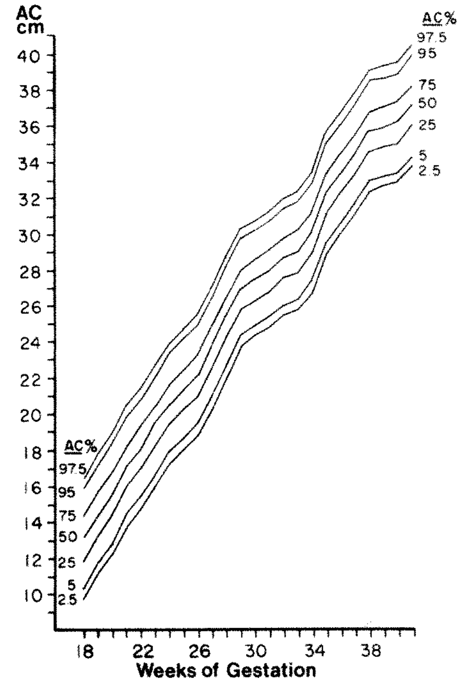

Fig. 3. Percentile ranks of fetal AC measurements in relation to gestational age.

cephalic growth was greater than or equal to the seventy-fifth percentile, indicating a younger fetus (Fig. 1), gestational age was reduced by 1 week.[8] Similarly, when cephalic growth was greater than or equal to the twenty-fifth percentile, indicating a more mature fetus[8] (Fig. 1), gestational age was advanced by 1 week. (3) Spontaneous delivery occurred at term in relation to the sonar EDD. (4) The neonate was found to be at term by pediatric assessment.[10]

A total of 238 BPDs and 238 ACs was obtained in the women with known menstrual dates and a total of 298 BPDs and 298 ACs was obtained in the women with ultrasonically defined dates according to the GASA chart[8]; this chart was shown to correlate with the composite mean BPD values derived from a large population of pregnant women.[11]

The BPDs were derived by either gray-scale or real-time imaging. All photographs selected showed a midline echo and symmetric skull tables, each 3 to 5 mm wide.[12] BPD values were derived from outer to inner (0 to 1) skull tables and measured in relation to 1 cm markers displayed on each photograph. The ultrasound velocity employed was 1,540 m/sec.

Two sonar steps were followed in obtaining all AC readings. First, the fetal longitudinal axis was outlined. Second, the transducer was positioned perpendicular to the fetal lie in the area just below the fetal heart, displaying the liver and umbilical vein (Fig. 2).

In 90 pregnant women the reproducibility of the AC was determined by comparing two photographs obtained independently from the upper fetal abdomen on different intervals of the same occasion. The photographs were sometimes derived by different technologists. In 110 pregnant women only one photograph of the AC was obtained. Each AC photograph was measured five times by a digitizer and the mean value was used as the accepted measurement.

In deriving the AC the digitizer pen was first passed over the outer perimeter of the AC (outlined by a pen, Fig. 2) and a digital readout was obtained. The digitizer pen was then passed over a defined number of markers constituting the 1 cm scale displayed on the photograph; in this way the scale was transformed into actual centimeters and the reduction ratio of the photograph was determined. For example, if 10 dots on the 1 cm marker scale equaled 3 cm by the digitizer, the scale reduction ratio would be 3/10, and fetal AC would equal digital readout × 10/3. The accuracy of sonar AC was determined by comparing the measurement to its anatomic counterpart in 11 fetuses within 24 hours after delivery by repeat cesarean section (Table II). The sonar AC values for each of the two groups were arranged in relation to gestational age at the time of each scan; the data were assumed to be normally distributed and percentile ranks were established for each group.

Results

The reproducibility of AC measurements obtained on different intervals of the same occasion fell within 2% of the mean. This range in error includes the variance obtained by the digitizer. The accuracy of the AC

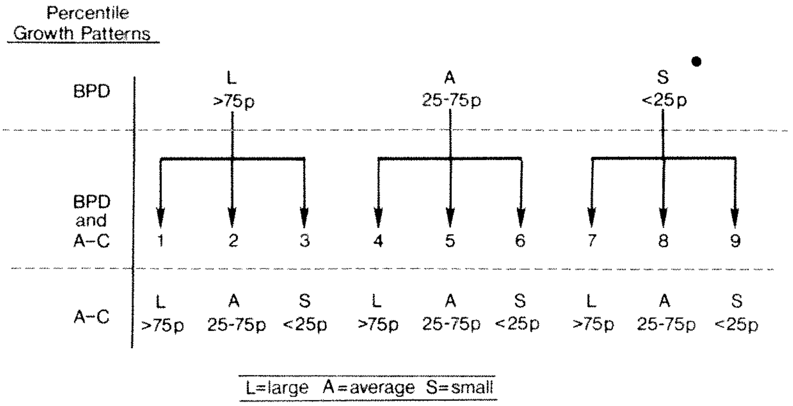

Fig. 4. Nine fetal growth patterns noted by using both BPD and AC percentile *(p)*.

Table III. Fetal AC measurements (in centimeters)*

Weeks of gestation	*Percentile*									*No.*
	2.5	*5*	*10*	*25*	*50*	*75*	*80*	*95*	*97.5*	
18	9.8	10.3	10.9	11.9	13.1	14.2	14.5	15.9	16.4	3
19	11.1	11.6	12.3	13.3	14.4	15.6	15.9	17.2	17.8	10
20	12.1	12.6	13.3	14.3	15.4	16.6	16.9	18.2	18.8	24
21	13.7	14.2	14.8	15.9	17.0	18.1	18.4	19.8	20.3	26
22	14.7	15.2	15.8	16.9	18.0	19.1	19.4	20.8	21.3	28
23	16.0	16.5	17.1	18.2	19.3	20.4	20.7	22.1	22.6	30
24	17.2	17.7	18.3	19.4	20.5	21.6	21.9	23.3	23.8	28
25	18.0	18.5	19.1	20.2	21.3	22.4	22.7	24.1	24.6	18
26	18.8	19.3	19.9	21.0	22.1	23.2	23.5	24.9	25.4	11
27	20.4	20.9	21.5	22.6	23.7	24.8	25.1	26.5	27.0	9
28	22.0	22.5	23.1	24.2	25.3	26.4	26.7	28.1	28.6	2
29	23.6	24.1	24.7	25.8	26.9	28.0	28.3	29.7	30.2	15
30	24.1	24.6	25.2	26.3	27.4	28.5	28.8	30.2	30.7	24
31	24.7	25.2	25.8	26.9	28.0	29.1	29.4	30.8	31.3	48
32	25.4	25.9	26.5	27.6	28.7	29.8	30.1	31.5	32.0	51
33	25.7	26.2	26.8	27.9	29.0	30.1	30.4	31.8	32.3	34
34	26.8	27.3	27.9	29.0	30.1	31.2	31.5	32.9	33.4	28
35	28.9	29.4	30.0	31.1	32.2	33.3	33.6	35.0	35.5	18
36	30.0	30.5	31.1	32.2	33.3	34.4	34.7	36.1	36.6	14
37	31.1	31.6	32.2	33.3	34.4	35.5	35.8	37.2	37.7	18
38	32.4	32.9	33.5	34.6	35.7	36.8	37.1	38.5	39.0	37
39	32.6	33.1	33.7	34.8	35.9	37.0	37.3	38.7	39.2	34
40	32.8	33.3	33.9	35.0	36.1	37.2	37.5	38.9	39.4	23
41	33.8	34.3	34.9	36.0	37.1	38.2	38.5	39.9	40.4	3

*Circumference measurements were obtained from the outer aspect of the fetal abdomen at the area of the liver which shows the umbilical vein.

measurement fell within 5.2% of the actual anatomic value (Table II).

The fetal AC percentile ranks from 18 to 41 weeks' gestation were determined separately for the following two groups: (1) 92 pregnant patients in whom gestational age was defined by menstrual data and (2) 108 pregnant patients in whom gestational age was defined by ultrasound data. The differences in the AC measurements of the two groups were not statistically significant ($\alpha = 0.05$), and the data were combined into one chart (Table III) and are depicted in Fig. 3. In addition the fetal AC percentile ranks from 18 to 41 weeks' gestation were compared in 40 black, 29 caucasian, and 23 Spanish women, all subgroups of the population of pregnant women where the length of pregnancy was defined by menstrual dates. Again the differences in these ACs were not statistically significant ($\alpha = 0.05$).

Comment

In this report we defined the percentile ranks of sonar-derived fetal AC values from 18 to 41 weeks' gestation. AC measurements were reproducible within a range of <2%. As such, fetuses with small ACs (less than twenty-fifth percentile) could be correctly discriminated from those with average (twenty-fifth to seventy-fifth percentile) or large (greater than eightieth percentile) values (Table III).

The accuracy of sonar AC values, i.e., their relationship to actual ACs (measured at the fetal xyphoid level after birth) was less precise and fell within a range of 5.2% (Table II). This discrepancy may be related to two factors. First, neonatal anatomic AC measurements were taken only during expiration, whereas the respiratory phase of the fetus at the time sonar AC was measured could not always be defined. Second, slight tightening or relaxation of the tape used in obtaining neonatal AC resulted in a difference of 0.5 to 1.0 cm.

It should be appreciated that the degree of accuracy of sonar AC is of no consequence in the assessment of fetal growth because sonar AC readings are reproducible to the extent of being useful as antenatal diagnostic parameters. Specifically, sonar fetal AC measurements add another dimension to the interpretation of BPD growth. For example, Sabbagha[13] showed that when serial BPDs fell in an upper or average percentile rank the risk of IUGR was 3.5% and 10%, respectively.[13] Additionally, he showed that the risk of IUGR was 52.1% in fetuses with small BPDs (less than twenty-fifth percentile). By evaluating the BPD and the AC percentile ranks for a given fetus, nine growth patterns emerge (Fig. 4); preliminary data indicate that: (1) fetuses falling in growth patterns Nos. 3 and 6 (i.e., normal BPD but small AC—less than twenty-fifth percentile) are at high risk for asymmetric IUGR; (2) fetuses falling in growth pattern No. 9 (i.e., BPD and AC are both small, less than twenty-fifth percentile) are at high risk for symmetric or near symmetric IUGR; and (3) macrosomic fetuses fall in growth pattern No. 1.[14]

Finally, knowledge of the fetal AC percentile rank relative to fetal age is significant for two reasons. First, it is useful in the assessment of fetal size in pregnant women who for obstetric reasons have to be delivered either in the latter part of the second trimester or early in the third trimester of pregnancy. In these women the AC may be helpful in defining the chances for fetal survival because it is a relatively close predictor of fetal weight (Table I),[15] a parameter of particular importance in the assessment of morbidity and mortality of low-birth weight neonates.[16] Second, the fetal AC is used as an adjunct in the diagnosis of some fetal abnormalities. For example, when hydrocephalus is suspected, the presence or absence of disproportion between the fetal head and body, ascertained by comparing BPD and AC percentile ranks, is important not only in establishing the diagnosis but also in delineating the severity of the condition. The severity and progression of fetal ascites also can be assessed by serial AC measurements.

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OBSTETRICS

Selective maternal culturing to identify group B streptococcal infection

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During a 5-year period vaginal cultures were obtained from all women with an obstetric history of premature onset of labor or premature rupture of membranes. With these indications, 1,213 (12.7%) of all parturient patients were cultured and 10.2% of those cultured were colonized with group B beta-hemolytic streptococci. Maternal colonization did not correlate with ABO blood group, although a significantly higher percentage of Rh negative women were colonized ($p < 0.01$). During this 5-year period, 10 infants had documented early-onset infection (sepsis or meningitis) with group B beta-hemolytic streptococci. All 10 infants had a maternal history of premature onset of labor and/or premature rupture of membranes. Mothers of eight infants were cultured and seven of these cultures were positive. Approximately one of every 20 infants designated at high risk actually developed early-onset disease. Selective maternal culturing effectively identifies those infants at risk for early-onset group B streptococcal disease. (AM. J. OBSTET. GYNECOL. 138:480, 1980.)

IN THE PAST DECADE group B beta-hemolytic streptococci (GBS) have become the major source of serious early-onset neonatal infection, with a mortality rate of greater than 50%.[1] As a result, intense efforts have been directed toward prevention and diagnosis of this fulminant disease. Recent attempts have centered on chemoprophylaxis of maternal carriers prior to or during labor and antibiotic therapy of neonates at the time of birth. These studies have been criticized on the basis of their enormous cost, the widespread antibiotic usage, and the lack of demonstrated efficacy.

An alternative approach would be the identification and treatment of only those infants at greatest risk of disease. Several studies have documented an increased rate of infection among infants with a history of obstetric complications, particularly premature onset of labor and prolonged rupture of membranes.[2, 3] For the past 5 years, the obstetric service at this hospital has obtained vaginal cultures at the time of admission from all women with onset of labor prior to 37 weeks' gestation or rupture of the membranes (ROM) without the onset of labor. This study entailed a retrospective analysis of these women and their infants to determine if this method of selective culturing would prove effective in identifying those infants at risk of serious infection.

Patients and methods

Patients studied were from the newborn and obstetric services of the Medical Center Hospital of Vermont in Burlington, Vermont. From October 1, 1974, through September 30, 1979, vaginal cultures were obtained from all women admitted to the labor floor with a history of onset of labor prior to 37 weeks'

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Received for publication March 24, 1980.
Revised July 3, 1980.
Accepted July 21, 1980.

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0002-9378/80/210480+05$00.50/0 © 1980 The C. V. Mosby Co.

gestation or ROM without onset of labor. Two culture swabs were obtained and transported immediately to the laboratory. One specimen was inoculated directly onto a selective blood agar medium. The second swab was processed in Todd-Hewitt broth. After 4 hours of incubation at 35° C an aliquot was streaked onto a blood agar plate containing phenylethylalcohol. Plates were read twice daily at 7:00 AM and 4:30 PM. All colonies identified as beta-hemolytic streptococcus were further typed according to the Lancefield classification.

The medical records of all women cultured were reviewed for duration of membrane rupture, gestational age, and blood type. The charts of all infants born to culture-positive women also were reviewed retrospectively for evidence of GBS infection or colonization. All other neonates with positive cultures for group B streptococci who were hospitalized during the study period were also subjected to review.

Results

Maternal colonization. During the 5-year period October 1, 1974, through September 30, 1979, vaginal cultures were obtained at the time of delivery from 1,213 women with an obstetric history of premature labor or premature ROM. They accounted for 12.7% of the 9,551 women who were delivered of live-born infants during this time period. One hundred twenty-four of the cultures were positive for group B streptococci, representing a colonization rate of 10.2%. The carrier rate was determined for each 6-month block during the study period and varied from 7.2% to 13.2% but with no recognizable trend.

There was no significant difference between ABO blood types of colonized and noncolonized women, in contrast to a previous report by Regan and associates[12] (Table I). Colonization rates were similar for women with B and other blood types and comparable rates were observed when B and AB blood types were considered together (Table II). A greater proportion of Rh negativity was seen among colonized as compared to noncolonized mothers (24% versus 14.9%, respectively) and likewise a greater percentage of Rh-negative women were colonized when compared to Rh-positive women (15.7% versus 9.3%).

Maternal age did not appear to be a factor associated with streptococcal carriage. The average age of colonized women was 25.6 years as compared to 25.3 years for noncolonized parturients.

Infant colonization. One hundred twenty-five infants were delivered to the 124 women with positive vaginal cultures for GBS. Eighty-two (66%) were asymptomatic during their hospital stay and were not cultured. One or more cultures were obtained from the remaining 43 infants. Nineteen of them (44%) had positive cultures at one or more sites. Superficial skin cultures were obtained from 12 infants, and eight were positive. Seven of 36 blood cultures (20%) were positive, and one of 25 cerebrospinal fluid cultures was positive. There were no positive urine cultures obtained from 13 specimens. There were four other positive cultures (throat 1, eye 1, and gastric aspirate 2).

Table I. Group B streptococcal colonization rates according to maternal blood type

	GBS negative		*GBS positive*	
Blood group	*No.*	*%*	*No.*	*%*
O+	432	39.9	48	38.4
O−	82	7.6	14	11.2
A+	373	34.4	36	28.8
A−	62	5.7	11	8.8
B+	87	8.0	9	7.2
B−	10	1.0	5	4.0
AB+	31	2.8	2	1.6
AB−	7	0.6	0	–
Rh+	923	85.1	95	76.0
Rh−	161	14.9	30	24.0

Table II. Relationship of group B streptococcal carrier rates to maternal blood groups

Blood group	*GBS carrier rates (%)*	*P value**
B + AB	10.6	NS†
O + A	11.5	
B	12.6	NS
O + A + AB	10.1	
Rh+	9.3	<0.01
Rh−	15.7	

*Chi-square test.
†Not significant.

Seven infants born to mothers who were cultured had evidence of systemic infection; six of these had positive blood cultures, and one had cerebrospinal fluid and blood cultures that were both positive for GBS.

Neonatal infection. During the 5-year study period, 31 infants admitted to the newborn service of MCHV had positive cultures for GBS within the first 5 days after delivery. The outcomes of these infants can be seen in Fig. 1. Eight infants were born at home or in outlying hospitals. Mothers of these infants were, therefore, not included in the study protocol. One infant had a positive blood culture at 4 days of age but a maternal vaginal culture was negative at the time of delivery. Neonatal blood cultures at 24 hours of age were also negative. Three infants were born to mothers who were not cultured at delivery. Two of these infants

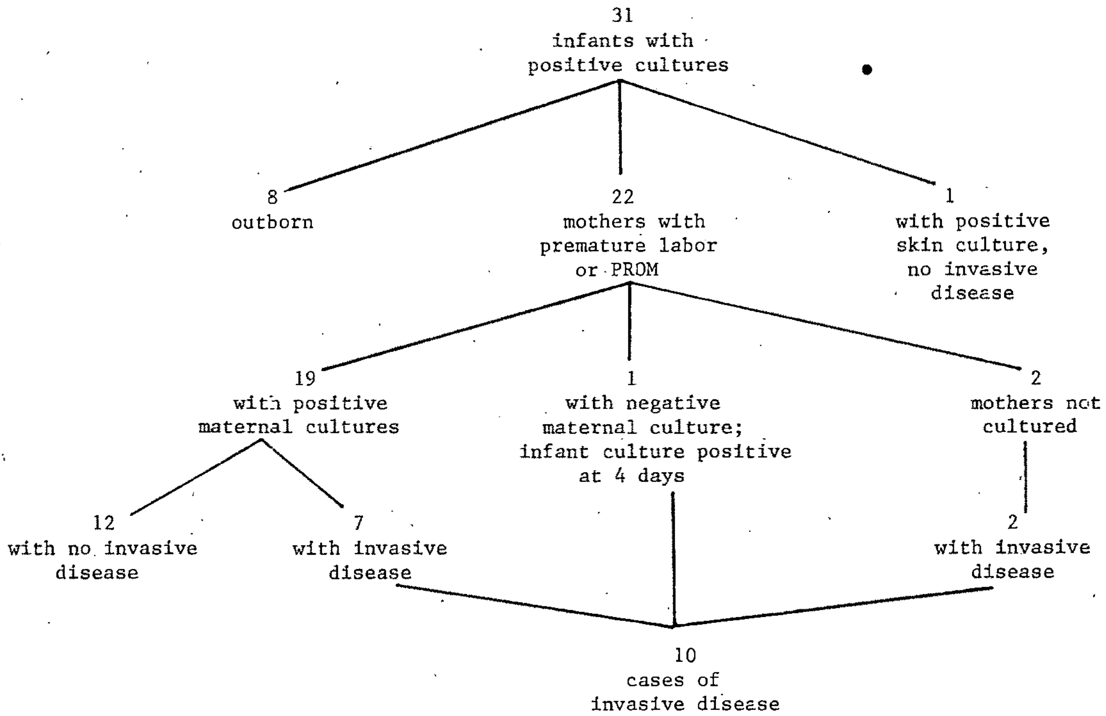

Fig. 1. Outcomes of neonates with positive cultures for group B streptococci.

were born prematurely and both had evidence of invasive disease. One mother had a precipitate delivery immediately following admission and before cultures could be obtained. The second mother was not cultured for unknown reasons. The third infant, born at term without premature ROM, had a positive skin culture but no evidence of invasive disease.

A total of 10 infants born in the hospital had documented invasive disease (sepsis or meningitis) during the study period, representing a rate of 10/9,551 or 1.04/1,000 live-born infants. Five of these infants died. All 10 infants were born to mothers who satisfied the criteria for obtaining vaginal cultures prior to delivery.

The average length of time between obtaining cultures and delivery was 10.3 hours for all positive cultures and 14.8 hours for those infants with invasive disease. Fifty-six percent of cultures were obtained more than 8 hours prior to delivery, and 95% were obtained more than 4 hours prior to delivery.

Duration of ROM correlated with the presence of invasive disease. In the 10 neonates born in the hospital with clinical GBS infection, 40% were associated with ROM greater than 24 hours, and only one was associated with ROM less than 12 hours. In those infants with positive maternal cultures but without invasive disease, 17% were associated with ROM greater than 24 hours while 40% were associated with ROM less than 12 hours.

Comment

The group B streptococcal organisms in early-onset disease are acquired from the maternal genital tract in utero or during passage through the birth canal. Recent studies have documented maternal colonization rates of 4% to 29% during the third trimester.[4] Despite this high carriage rate, the incidence of neonatal infection is relatively low, at 1.3 to 5.4/1,000 live births or approximately one of every 100 infants born to colonized mothers.[4, 5]

Epidemiology. The epidemiologic data obtained in this study generally concur with the findings of others. The maternal carrier rate of 10.3% is within the range observed in other studies. Although the women cultured cannot be considered representative of the population as a whole, the majority of studies have shown no correlation between vaginal colonization rates and the presence of obstetric complications.[1] Therefore, it is reasonable to assume that a carrier rate of 10.3% is characteristic of this hospital population. The disease attack rate of 1.04 per 1,000 live births is similar to that in earlier reports, whereas the ratio of colonized mothers to infected babies of 124:7 (or less than 20:1) is considerably lower than previously reported.[1]

Prevention. Because of the rapid and frequently fatal course of early-onset GBS infections, emphasis has been placed on prevention and early diagnosis. Several recent studies have attempted to decrease the incidence of early-onset disease by eradicating maternal colonization during pregnancy with prophylactic antibiotic therapy.[2, 6–8] Three major criticisms have been leveled against this approach.

1. Because of the marked discrepancy between maternal colonization rates and the attack rate of neonatal disease, approximately 100 women would receive antibiotic prophylaxis to prevent one case of neonatal infection. This not only entails an enormous cost but exposes a large number of women to the risks of antibiotic therapy as well.

2. No randomized, controlled evidence exists that such management would reduce the incidence of neonatal disease. Several studies have questioned the efficacy of antibiotic therapy in eradicating colonization; two recent studies demonstrated the failure of oral penicillin to reduce maternal colonization at the time of delivery.[7, 8]

3. The estimated cost of culturing all women in the third trimester and treating up to 25% with a full course of antibiotic therapy would be enormous. (In this hospital, the cost of obtaining vaginal cultures on all women prior to delivery would be $31,040 per year. By the selective culturing technique used in this study, the cost would be reduced by 87%, to $4,035 per year.)

Other studies have recommended antibiotic prophylaxis of all infants at the time of delivery.[9, 10] However, this would entail exposing large numbers of noninfected infants to unnecessary antibiotics. In addition, at least one study has documented the failure of single-dose penicillin to eradicate colonization in the newborn infant.[11]

Identification. An alternative approach to these problems would be the identification of risk factors for early-onset disease and treatment of only those infants at high risk. An increased incidence of maternal obstetric complications, particularly premature onset of labor or prolonged ROM, has been observed to be a risk factor for development of early-onset infection. Baker and Barrett,[3] in 1973, documented an 80% incidence of low birth weight (<2,500 grams) and a 62% incidence of ruptured membranes greater than 24 hours in 13 infected newborn infants. These findings were the basis of our prenatal screening program to identify those infants at greatest risk of disease. During the 5-year study period, 10 infants born in the hospital had early-onset GBS sepsis diagnosed. Data on these infants are similar to those observed by Baker and Barrett, with a 70% incidence of birth weight less than 2,500 grams and a 40% incidence of ROM greater than 24 hours. All infants born in the hospital with GBS sepsis were delivered of women who fit the criteria for inclusion in this study, i.e., onset of labor at less than 37 weeks' gestation or rupture of the membranes without onset of labor.

A maternal risk factor was proposed by Regan and associates,[12] in 1978, when they documented an increased incidence of blood type B among colonized women. They hypothesized that GBS organisms possess a B-like antigen and that individuals who lack anti-B antibody are at increased risk of colonization. In our study of 1,213 parturients, we failed to demonstrate a significant difference in blood type among those women colonized or not colonized with GBS. However, a discrepancy was observed in our data concerning Rh negativity. Twenty-four percent of colonized mothers were Rh negative as opposed to 14.9% of noncolonized women, and, similarly, a greater percentage of Rh-negative women were colonized when compared to Rh-positive women (15.7% versus 9.3%). The distribution frequency of neonatal blood type was not evaluated because of the small number of infants involved. Further studies are needed to confirm and elaborate on these findings.

The average length of time from obtaining cultures to delivery was 14.8 hours for mothers of infected infants. At the time of this study, positive cultures were rarely identified in less than 24 hours. However, by means of a selective Todd-Hewitt medium in conjunction with coagglutination techniques, reports of positive cultures can be returned to the physician within 8 hours of inoculation.[13] Results of maternal cultures could, therefore, be available for 95% of infants within 4 hours of birth.

This method of selective maternal culturing identified seven of eight infants born in the hospital who subsequently developed early-onset infection. Two infants whose mothers were not cultured also had GBS disease; however, these cases must be considered as potentially avoidable rather than inherent methodologic failures. During this 5-year period, only one infant who developed GBS disease was born to a mother with a negative streptococcus screening test at delivery. This infant had an initially negative blood culture at 24 hours of age but developed GBS sepsis on the fourth day of life. It is possible that this infection resulted from acquisition of bacteria following delivery. GBS nosocomial colonization of infants born to noncolonized mothers has been documented in several nurseries.[14, 15]

Another infant with a positive GBS culture was delivered during the study period of a mother who was not included in this selected population. This infant was colonized but not infected with the GBS organism. Blood, urine, and cerebrospinal fluid cultures were negative. Since only those mother-infant pairs with a history of obstetric complications were included in this study, it is probable that the majority of colonized infants were not identified. However, as noted previously, all infected infants born in the hospital had a maternal history of such obstetric complications.

Conclusions

1. This protocol identified seven of eight infants born in the hospital who developed early-onset GBS disease during the 5-year study period. By means of new culture techniques, the majority of high-risk infants can be identified at birth or shortly thereafter. This method of selective maternal culturing is an effective means of identifying infants at high risk for early-onset GBS disease.

2. With the use of the selective indications of premature onset of labor or premature ROM, only 12.7% of the parturient population was cultured. This resulted in a significant cost reduction over previously recommended protocols.

3. This study did not confirm the previous finding of an increased incidence of blood type B among colonized mothers.[12] A significantly greater percentage of Rh-negative women were colonized when compared with Rh-positive women.

4. The ratio of infants at risk to infants infected with GBS has previously been reported at 100: 1.[4] This selective maternal culturing protocol enabled us to decrease this ratio to 20: 1, thereby achieving substantial savings in effort and expense without sacrificing sensitivity of the screening test.

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Stimulus summation and tachyphylaxis in estrogen response in sheep

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The response of the uterine circulation to estrogen has been extensively studied. Estrogen is the most potent vasodilator of the uterine bed. The magnitude of the vascular response depends on both the dose of estrogen used and the duration of exposure to estrogen. In this study, the relationship between the arterial concentration of estrogen, the duration and pattern of exposure, and the response was studied. This is a complex relationship, but one that is consistent with accepted models of estrogen-target tissue interaction. It was also shown that prolonged, high-concentration exposure results in tachyphylaxis. (AM. J. OBSTET. GYNECOL. 138:485, 1980.)

IN EACH AREA of physiology, studies of stimulus-response relationships have followed unique lines of development by investigation in great detail of those aspects which are more readily controlled and measured. In neurophysiology, the characteristics of excitable membranes have been thoroughly analyzed by means of electric stimuli and responses. However, the underlying biochemical mechanisms remain obscure. The understanding of stimulus-response relationships in the endocrinologic activities of steroids has developed in a different way. The measured reponse has usually been an anatomic change or biochemical effect measured at relatively few time points after application of stimulus. Although this limits studies of response kinetics, the more serious methodologic limitation has been quantitation and control of the stimulus.

Most studies have involved systemic hormonal doses. This type of stimulation has uncertain characteristics because its time course depends upon absorption rates, distribution kinetics, and metabolism. For these reasons, physiologic studies of stimulus response in the endocrinologic activities of steroids have been relatively crude. In contrast, the biochemical response to steroid hormones has been much more amenable to study and has revealed an intricate mechanism. Specificity is conferred on hormonal response by the presence of soluble receptors in the cytoplasm of target cells.[1] Combination of the steroid with its receptor molecule is followed by translocation of the receptor-hormone complex to the nucleus, where the complex interacts with nuclear chromatin. This interaction initiates the transcription of ribosomal and messenger ribonucleic acid.[2] New protein is then synthesized within the responding cell, and leads to new cell products or cell growth and division. This sequence of events has been most thoroughly studied in the chick oviduct.[3] This knowledge suggests that the stimulus-response kinetics of steroid hormones would be complex and physiologically significant. For the reasons stated above, however, physiologic kinetic studies would be difficult in the systems most used for the investigation of steroid action.

The induction of uterine vasodilatation by estrogens in castrated sheep is more amenable to kinetic studies than other examples of steroid stimulation. Although this response has not been so thoroughly studied biochemically as the response of the chick oviduct, indirect data suggest that a similar sequence of events is involved.[4] The response, uterine vasodilatation, can be measured continuously by blood flow transducers placed around the uterine arteries. The stimulus, estrogen, can be administered by intra-arterial injection in precisely controlled amounts over a specified period of time. Thus, the intensity and duration of stimulation can be determined and varied as desired. Furthermore, since the circulations of the two uterine horns of the

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Supported by United States Public Health Service Grants HD 00781 and HD 01866 and by Training Grant HD 00429.

Received for publication April 23, 1980.

Accepted July 22, 1980.

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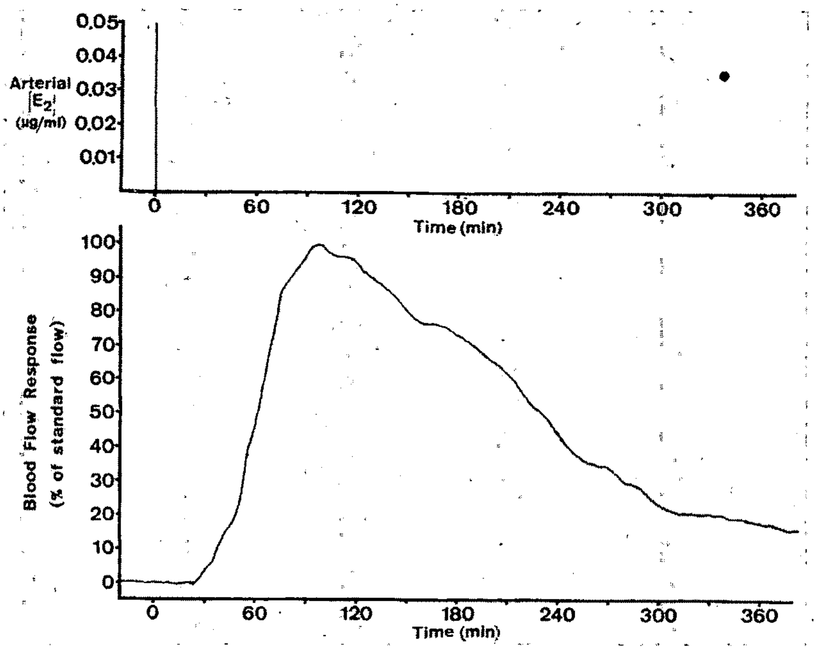

Fig. 1. Blood flow response to the intra-arterial injection of 1 µg of 17β-estradiol (E_2) over 1 minute. The response is expressed as percent of mean maximal change in flow after 1 µg of E_2 injected over 1 minute (standard flow).

sheep are functionally independent, each animal serves simultaneously as experiment and control.[5]

In this study, the stimulus-response relationship in estrogen-induced uterine vasodilatation was investigated. As expected, the relationship was complex and had features of physiologic interest.

Material and methods

Eight mixed-breed nonpregnant ewes were studied. The techniques for measurement of uterine artery blood flow used in this study were described previously.[5] After bilateral oophorectomy, an electromagnetic flow transducer was placed around each main artery proximal to its first branch within the broad ligament of the uterus. A polyvinyl catheter (internal diameter, 0.38 mm; outer diameter, 1.09 mm) was inserted into a branch of each uterine artery and secured by a ligature and tissue cement (Eastman 910 adhesive, Eastman Chemical Products, Inc., Kingsport, Tennessee). Flow transducer leads and uterine arterial catheters were brought through the fascia and a subcutaneous tunnel to a pouch on the animal's flank. A third polyvinyl catheter was threaded into the inferior vena cava via the left femoral vein, secured, and brought to the flank pouch by a subcutaneous tunnel.[5]

A concentrated stock solution of 17β-estradiol (100 µg/ml) (Sigma Chemical Company, St. Louis, Missouri) was prepared by dissolving the estrogen in the solvent (40% propylene glycol, 10% ethanol, 50% physiologic saline solution) on a water bath at 85° C. A dilute solution of 17β-estradiol (10 µg/ml) was prepared by diluting the stock solution with physiologic saline solution and heparin (100 units/ml). The solvent of the dilute solution (4% propylene glycol, 1% ethanol, 95% physiologic saline solution with heparin) caused no vascular response in the uterine artery.

In order to expose the uterine vasculature to precise quantities of estrogen during a defined time interval the intra-arterial infusions were made by displacement. Known volumes of estrogen solution were injected into the catheter by a syringe pump (Sage Instruments Model 355 variable speed syringe pump, Orion Research Inc., Cambridge, Massachusetts) displacing an equivalent quantity of estrogen into the uterine artery. The polyvinyl catheters were "seasoned" with estrogen solution during the first 5 postoperative days. They were filled with solution each day and allowed to equilibrate in order to prevent loss of estrogen to the catheter by absorption during the experimental period. The small diameter of the catheter assured minimal intraluminal dilution and mixing at the catheter tip. The venous catheter was flushed with heparinized saline solution daily and after all injections of 17β-estradiol in order to maintain patency.

Test doses of 17β-estradiol were infused into each uterine artery beginning on the sixth postoperative

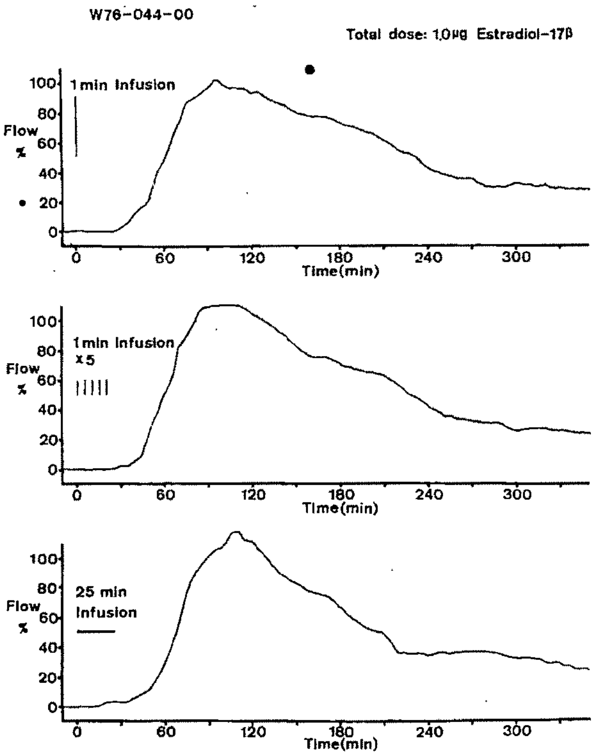

Fig. 2. Comparison of the responses, in a single animal, to intra-arterial injection of 1 μg of E_2 in three different ways. Flow is expressed as percentage of standard flow.

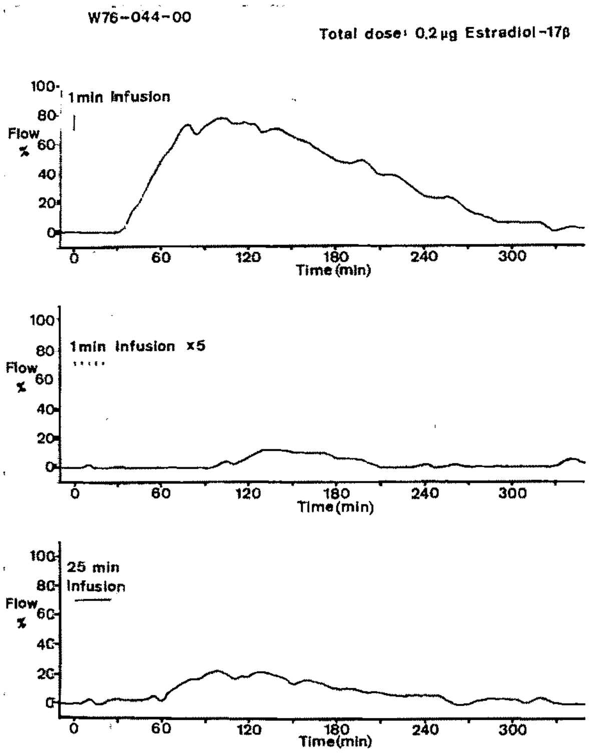

Fig. 3. Comparison of the response, in a single animal, to the intra-arterial injection of 0.2 μg of E_2 in three different ways. Flow is expressed as percentage of standard flow.

day. Three modes of administration of estrogen were used: (1) single injections of estrogen with the total dose given over 1 minute, (2) divided doses with five 1-minute infusions at 5-minute intervals, and (3) continuous infusion with the total dose given over 25 minutes. Six different total doses were used (0.05, 0.10, 0.20, 0.40, 0.80, and 1.0 μg). These six doses combined with three different modes of administration resulted in 18 different dose-mode combinations. Each animal was exposed to all 18 doses in a randomized order.

Preliminary experiments demonstrated that the uterine blood flow response on a given day depended, to some extent, on the estrogen dosage given on the previous day. Consequently, a maximally stimulating, systemic dose of 17β-estradiol (50 μg) was given intravenously daily between 1600 and 1700 hours in order to minimize the variability of responsiveness to the test doses of estrogen.

The dose-response curves for the various modes of stimulation with estrogen were compared by means of a two-way analysis of variance. Five animals were used in the comparison of dose-response curves. Since flow data were not available for both uterine arteries at all dose-mode combinations, the responses of right and left sides were meaned in all animals. Thus, for each dose there were three modes of administration given to five animals.

The effects of long-term infusions of 17β-estradiol were studied by infusion of the estrogen into one uterine artery and of the solvent into the other for 48 to 72 hours. Infusion rates of 2, 4, and 8 μg/hr were used. At rates above 8 μg/hr, vasodilatation was noticed in the contralateral horn. Vascular responsiveness to 17β-estradiol during long-term infusions was tested with intermittent intravenous injections of 17β-estradiol in maximally stimulating doses (50 μg). Uterine horns made nonresponsive to 17β-estradiol were challenged with bradykinin (bradykinin triacetate, Sigma Chemical Company, St. Louis, Missouri) and adenosine (J. T. Baker Chemical Co., Phillipsburg, New Jersey). Adenosine solution was prepared by dissolving adenosine in saline solution (64 μg/ml, 0.24 mM/L). Bradykinin solution was prepared immediately prior to use from fresh crystals of bradykinin (1.24 μg/ml, 1 μmole/L) dissolved in sterile saline solution. Infusions were made by syringe pump via the uterine artery catheters.

Results

Uterine blood flow, as a function of the time after a single 1-minute infusion of 17β-estradiol into the uter-

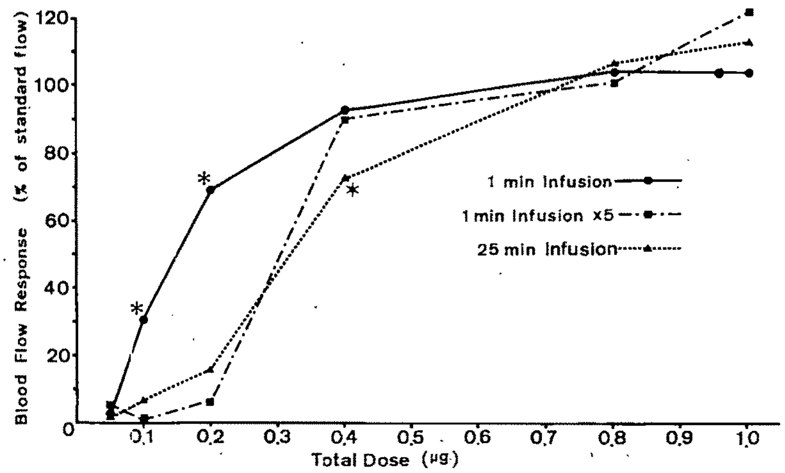

Fig. 4. Dose response curves for intra-arterial E_2 injected in three different ways. Flow is expressed as percentage of standard flow. The curves are significantly different ($P < 0.05$) at the 0.1, 0.2, and 0.4 μg dose points. Significance was tested by two-way analysis of variance, with $V_1 = 2$ and $V_2 = 8$ for all points.

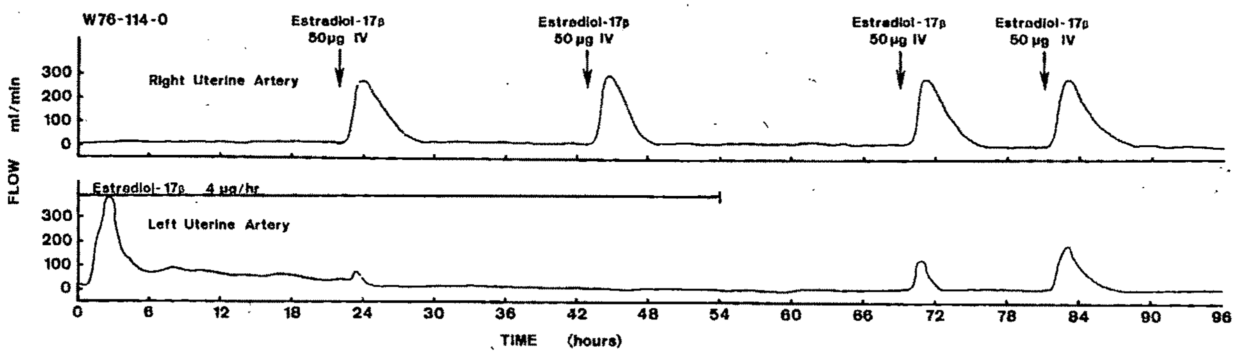

Fig. 5. Response of uterine circulation to continued infusion of estrogen. E_2 is infused at 4 μg/hr for 54 hours into the left uterine artery. Responsiveness of uterine vasculature is tested, at time indicated, by intravenous injection of E_2, 50 μg.

ine artery, is shown in Fig. 1. For the first 30 minutes after infusion, there was no change in uterine blood flow. Then there was a vasodilatation which was maximal at approximately 2 hours. Over the next 6 hours the flow returned to baseline. By confining all variations in the mode of administration to the first 30 minutes, the effect of increasing blood flow on arterial concentration was avoided. The uterine blood flow responses to a total dose of 1 μg given as a single 1-minute infusion, five 1-minute infusions at 5-minute intervals, and a 25-minute infusion are shown in Fig. 2. There were no significant differences in the magnitude or the time course of the response between the three modes of administration for this dose.

Fig. 3 shows uterine blood flow as a function of the time after a total dose of 0.20 μg of 17β-estradiol for the three modes of administration. For the bolus infusion, the peak flow response to 0.20 μg of 17β-estradiol was approximately 80% of the response obtained from 1 μg. The peak flow response to 0.20 μg, given in divided doses or as a 25-minute infusion, was only 10% to 20% of the maximal response to a bolus.

Dose-response curves for the three modes of administration of estrogen are illustrated in Fig. 4. Blood flow response was expressed as a percentage of the response to 1 μg of 17β-estradiol injected into the uterine artery over 1 minute (standard flow). The curves were significantly different at the 0.1-, 0.2-, and 0.4-μg dose points ($P < 0.05$). There were no significant differences in blood flow responses between the three modes of administration at the other dosages tested.

Uterine blood flow versus time during a continuous infusion of 17β-estradiol (4 μg/hr) into one uterine artery and of solvent into the contralateral artery is

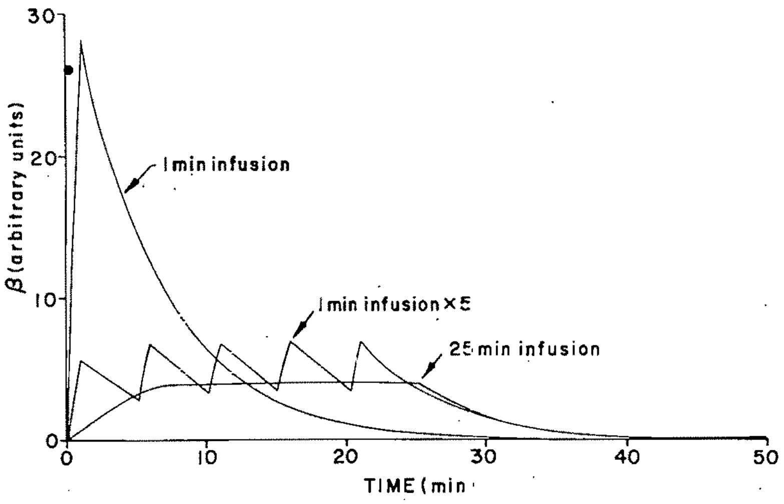

Fig. 6. Time-course of concentration of cytosol estrogen-receptor complex (β) as postulated by the mathematical model. The three modes of injection of the same dose generate different concentration patterns.

shown in Fig. 5. After an initial maximal vasodilatation on the side which received the estrogen solution, the blood flow decreased gradually toward baseline. A second smaller peak of flow then occurred, approximately 8 hours after initiation of the infusion, followed by a gradual return to baseline. The responses of blood flow to infusions of 2 and 8 μg/hr of 17β-estradiol were similar. At infusion rates greater than 8 μg/hr, vasodilatation was noticed in the contralateral horn.

The responsiveness of the uterine vasculature to a maximally stimulating bolus dosage of 17β-estradiol (50 μg) administered systemically was tested periodically during the continuous infusions after flows had returned to baseline (Fig. 5). Uterine horns which received the infusion of solvent showed the characteristic maximal vasodilatation after the systemic injections of 17β-estradiol. However, the uterine horns infused with 2 μg of 17β-estradiol showed a blunted response to systemic injections of 17β-estradiol, whereas those infused with 4 or 8 μg/hr of 17β-estradiol were unresponsive to the systemic dose after 48 hours of infusion. The responsiveness of the uterine vasculature to large systemic dosages of 17β-estradiol decreased gradually during the infusion. The response returned toward normal within 24 hours after the intra-arterial infusion of 17β-estradiol was stopped (Fig. 5).

During the period of vascular refractoriness to 17β-estradiol, the estrogen-infused and solvent-infused horns showed equal magnitudes of vasodilatation to intra-arterial infusions of adenosine and bradykinin.

Comment

The increase in uterine blood flow after the intra-arterial injection of a bolus of estrogen is a reproducible phenomenon whose characteristics have been defined previously.[5] The flow begins to increase approximately 25 minutes after the injection, reaches its maximum at circa 100 minutes, and then decreases slowly toward baseline level over several hours (see Fig. 1).

In order to analyze the stimulus that produces this response, consider first the hypothesis that the only relevant aspect of the stimulus is the concentration of estrogen in the blood, and that the exposure time to a given concentration is of no consequence. According to this hypothesis, a dose of 0.8 μg infused over a period of 25 minutes should have no more effect than a dose of 0.032 μg injected over 1 minute. The inadequacy of this hypothesis is clearly shown in Fig. 4. It can be seen that in the 1-minute bolus injection modality, 0.032 μg causes virtually no response, whereas exposure of the organ to a constant infusion of 0.032 μg per minute over a period of 25 minutes (0.8 μg total) causes a maximal response. Thus, the response depends upon the duration of the stimulus. This conclusion leads to testing an alternative hypothesis, namely, that the relevant stimulus is the concentration × time product. In this case, we would expect equal response for equal total dose, irrespective of the time period over which the dose is administered. The uterine blood flow response to estrogen does not fit this simple model because at submaximal doses the rapid stimulation by bolus injection is much more effective than the infusion or divided-dose modality (see Fig. 4).

A variety of hypotheses could be invoked to explain this complex stimulus-response relationship. In the case of steroids, the best established and most thoroughly studied model for hormone action is that proposed by Williams and Gorski.[8] In this model, estrogen

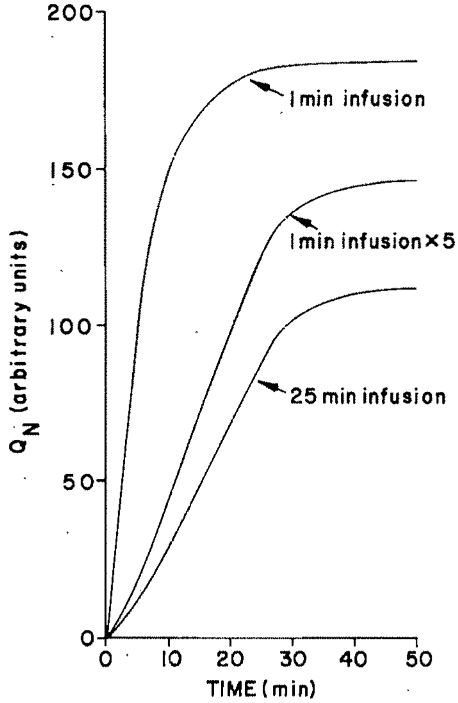

Fig. 7. Time-course of accumulation of estrogen in uterine nuclei with each mode of injection of the same dose. Data derived from the mathematical model.

interacts with the target tissue in two steps: (1) binding to cytoplasmic receptor proteins; (2) translocation of this complex into the nucleus, where the estrogen is bound to the chromatin.

The data on estrogen-induced uterine vasodilatation are compatible with this model. The 20- to 30-minute lag between the stimulus and the beginning of the response is adequate to allow the synthesis of new peptide. The response is completely blocked by cycloheximide, an inhibitor of protein synthesis. Although the increase in blood flow after estrogen stimulation could not be inhibited by actinomycin D[4], ribonucleic acid synthesis could not be completely inhibited, so that the dependence of the response on a transcriptional step has not been ruled out. Other models that involve the release of histamine, catecholamines, acetylcholine, or prostaglandins do not seem to be so fruitful at the present time because potent antagonists of these agents have failed to block the response.[6, 7] Thus, we have chosen to make a tentative explanation of our data in terms of the Williams and Gorski model. Some aspects of this explanation are amenable to further testing.

If the binding of estrogen to cytoplasmic receptors is more rapid than the translocation into the nucleus, then the cytoplasmic receptors function as temporary sites for estrogen storage. We postulate that at submaximal physiologic doses the receptors are unsaturated and, therefore, store the hormone in a quantity which is proportional to the concentration of free estrogen in the cytoplasm at the end of the infusion. Larger storage at higher concentrations of estrogen in the blood explains the advantage of rapid stimulation by bolus injection. The ability of the organ to act as a time integrator of a low concentration of estrogen is explained chiefly by the fact that the nucleus removes the estrogen-receptor complex from the cytoplasm. Thus, a steady concentration of estrogen in plasma results in a steady flux of estrogen from cytoplasm to nucleus. The quantitative aspects of these concepts are presented in the Appendix. A mathematical model was constructed on the basis of the assumption that equivalent physiologic responses are evoked by equivalent amounts of estrogen accumulated in the nucleus. According to the model, the three modalities of stimulation described in this report produce distinctly different time-courses of estrogen-receptor complex in the cytoplasm (Fig. 6), and, consequently, different patterns of nuclear accumulation (Fig. 7). The quantity of estrogen entering the Nucleus $(Q_N)_{tot}$ during and after an infusion lasting t minutes is given by the equation:

$$(Q_N)_{tot} = C\epsilon_p[t + 3(1 + e^{-0.33t})] \qquad (1)$$

where C is a coefficient and ϵ_p is the concentration of free estrogen in plasma. The number 3 represents the difference, in minutes, between the time constant for the removal of the estrogen-receptor complex from the cytoplasm (6 minutes) and the time constant for attaining a steady state relationship of estrogen concentrations between blood and cytoplasm (3 minutes). The time-courses of bound estrogen in cytoplasm and nucleus which are predicted by the model are in qualitative agreement with the experimental data of Williams and Gorski[8] on uterine estrogen-receptor kinetics. Those authors have shown that the exposure of rat uteri in vitro to a constant concentration of estrogen produces after a few minutes a steady concentration of bound estrogen in the cytoplasm and a linear increase in bound estrogen in the nucleus.

According to Equation 1, a prolonged exposure of the target organ to a steady concentration of estrogen in the blood would result in a continuous entry of estrogen into the nucleus, the rate of which depends on the magnitude of the C coefficient. In order to discuss what physiologic effects may result from such prolonged exposure, we must consider the relationship of Q_N to the physiologic effect and the fact that the magnitude of the C coefficient depends upon the concentration of cytoplasmic estrogen receptors (see Appendix, Equation 14A). The uterine blood flow response to estrogen has a relatively short life. Hence, the production of a measurable flow response requires, presumably, that the rate of entry of estrogen into the target cell exceed some minimum rate. Therefore, in addition to a minimum $\epsilon_p \times t$ product for the response, there is probably a threshhold value of ϵ_p below which no effect ensues even for large values of the $\epsilon_p \times t$ product. A consideration of the effect of exposing the organ to large concentrations of estrogen for a prolonged period of time must recognize the fact that the process by which estrogen receptors are replenished is relatively slow, so that large doses of estrogen can deprive the uterus of receptors.[9] Therefore, the continuous infusion of estrogen may lead to virtual absence of receptors in the cytoplasm and may explain the tachyphylaxis which we describe in this report (see Fig. 5). Likewise, the slow return toward a normal response after cessation of a prolonged infusion of estrogen may represent a replenishment of estrogen receptors.

Knowledge that the modality of estrogen stimulation has a profound effect on the physiologic response is of interest because a great variety of plasma estrogen concentration patterns has been described in association with distinctly different physiologic states. These patterns may be an essential component of the signal to the target organ, which "interprets" each pattern differently in accordance with the kinetic properties of its receptors.

The uterine vasodilatation response to estrogen is a system in which both the stimulus (intra-arterial estrogen) and the response (vasodilatation and increased blood flow) can be precisely controlled and measured. We have shown that the magnitude of the response depends on the arterial concentration of estrogen and duration and pattern of exposure to estrogen. We developed a mathematical model that explains this complex stimulus-response relationship in terms of current biochemical concepts of the action of estrogen. The model suggests that, by variations in the quantity of cytosol receptor, its rate of synthesis, and rate constant for the entry of estrogen-receptor complex into the nucleus, the responding organ can be made to be particularly sensitive to very brief pulses of estrogen, or lower concentration but prolonged estrogen stimulation. Thus, estrogen-sensitive tissues may be able to differentiate between varying patterns of estrogen stimulation with qualitatively as well as quantitatively different responses.

Physiologic effects of different patterns of hormonal stimulation have been described for gonadotropin-releasing hormones, luteinizing hormone, catecholamines, and insulin.[10] In these types of hormonal stimulation, the phenomenon of "down regulation" has been attributed to a decline in the number of hormone receptors on the surface of responding cells. Intermittent exposure results in dramatic responses. Continuous exposure results in refractoriness. This is very similar to our observations, which represent the first demonstration of such an effect with steroid hormones.

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Appendix

List of symbols.

Dose = quantity of estrogen infused into the artery (μg).

F = baseline arterial flow before the response (ml · min^{-1}).

K_A = association constant between ϵ and R (ml · μg^{-1}).

K_1 = diffusion constant (ml · min^{-1}).
K_2 = diffusion constant of β into the nucleus (ml · min^{-1}).
K_3 = rate constant for the removal of bound estrogen from cytoplasm (ml · min^{-1}).
$\dot{Q}_{cell}$ = rate of entry of estrogen into cell (μg · min^{-1}).
$\dot{Q}_N$ = rate of entry of estrogen into nucleus (μg · min^{-1}).
Q_N = quantity of estrogen present in the nucleus at a given time (μg).
$(Q_N)_{tot}$ = total quantity of estrogen entering the nucleus (μg).
R = concentration of estrogen receptors in cytoplasm (μg·min^{-1}).
t = time during the infusion (min).
t′ = time after the infusion (min).
t_{inf} = time at which the infusion is stopped (min).
t_o = beginning of infusion (min).
V = volume of cytoplasm (ml).
β = concentration of bound estrogen in cytoplasm (μg · ml^{-1}).
β_t = concentration of bound estrogen in cytoplasm at time t.
ϵ_p = concentration of free estrogen in arterial plasma (μg · ml^{-1}).
ϵ = concentration of free estrogen in cytoplasm (μg · ml^{-1}).
ϕ = fraction of blood estrogen which is free.

During the infusion of estrogen into the uterine artery, the rate of entry of estrogen into the target cell is proportional to the concentration difference of free estrogen between arterial plasma and cytoplasm:

$$\dot{Q}_{cell} = K_1(\epsilon_p - \epsilon). \qquad 1A.$$

Neglecting nonspecific binding, this rate of entry is the sum of the accumulation rates of free and receptor-bound estrogen in cytoplasm and nucleus:

$$\dot{Q}_{cell} = V\left(\frac{d\epsilon}{dt}\right) + V\left(\frac{d\beta}{dt}\right) + \dot{Q}_N. \qquad 2A.$$

We assume that, in the dose range that causes submaximal flow responses, the formation of the cytoplasmic estrogen-receptor complex β utilizes only a small fraction of the available receptors, and that the equilibrium between ϵ and β is established rapidly. Thus, as a first approximation:

$$\beta = \frac{K_AR\epsilon}{1 + K_A\epsilon} = K_AR\epsilon. \qquad 3A.$$

Furthermore, we assume that the rate of entrance of estrogen into the nucleus is proportion to β:

$$\dot{Q}_N = K_2\beta. \qquad 4A.$$

Combination and rearrangements of Equations 1, 2, 3, and 4A provide the following relationship:

$$\frac{d\beta}{dt} = a\epsilon_p - b\beta \qquad 5A.$$

where

$$a = \frac{K_1K_AR}{V(1 + K_AR)} \qquad 6A.$$

and

$$b = \frac{K_1 + K_ARK_2}{V(1 + K_AR)} \qquad 7A.$$

During the intra-arterial infusion, ϵ_p is constant. Therefore, integrating Equation 5A:

$$\beta_t = \frac{a}{b}\epsilon_p(1 - e^{-bt}). \qquad 8A.$$

At the moment the infusion is terminated, β begins to decrease exponentially because of transport into the nucleus and dissociation into free receptor and estrogen:

$$-V\frac{d\beta}{dt'} = K_3\beta. \qquad 9A.$$

Since at the end of the infusion

$$\beta = \frac{a}{b}\epsilon_p(1 - e^{-bt_{inf}}), \qquad 10A.$$

it follows that, at time t′ after the end of the infusion, β is defined by the equation:

$$\beta_{t'} = \frac{a}{b}\epsilon_p(1 - e^{-bt_{inf}})\,e^{\frac{-K_3t'}{V}}. \qquad 11A.$$

The quantity of estrogen entering the nucleus, Q_N, is related to β:

$$Q_N = K_2\int_{t_o}^{t_{inf}}\beta_t dt + K_2\int_{t_{inf}}^{t'} b_{t'}dt', \qquad 12A.$$

i.e.,

$$Q_N = \frac{a}{b}K_2\epsilon_p\left[\left(t - \frac{(1 - e^{-bt})}{b}\right) + \frac{V}{K_3}(1 - e^{-bt_{inf}})\left(1 - e^{\frac{-K_3t'}{V}}\right)\right]. \qquad 13A.$$

Therefore, the total quantity of estrogen $(Q_N)_{tot}$ entering the nucleus during and after the infusion is related to ϵ_p and t_{inf} as follows:

$$(Q_N)_{tot} = \frac{a}{b}K_2\epsilon_p\left[t_{inf} + \left(\frac{V}{K_3} - \frac{1}{b}\right)(1 - e^{-bt_{inf}})\right] \qquad 14A.$$

Since

$$\text{Dose} = \frac{\epsilon_p \cdot F \cdot t_{inf}}{\Phi}, \qquad 15A.$$

$(Q_N)_{tot}$ is related to the dose by the equation:

$$(Q_N)_{tot} = \frac{a\Phi K_2}{bF}\,\text{Dose}\left[1 + \frac{\left(\frac{V}{K_3} - \frac{1}{b}\right)(1 - e^{-bt_{inf}})}{t_{inf}}\right]. \qquad 16A.$$

The latter equation shows that the case in which $(Q_N)_{tot}$ is a function of the dose, irrespective of infusion time, would occur only if the time constants $\frac{V}{K_3}$ and $\frac{1}{b}$ were equal or estrogen would combine slowly with cytoplasmic receptors (i.e., if b were a small number). If $\frac{V}{K_3} > \frac{1}{b}$ and formation of the estrogen-receptor complex in the cytoplasm is rapid, $(Q_N)_{tot}$ is maximal when the dose is infused over a very short period of time. Given the fact that this same relationship exists between flow response and infusion time, it seems reasonable to postulate that the flow response is determined by $(Q_N)_{tot}$. On the basis of the data provided by the 1-minute and 25-minute infusion modalities, there is not enough information to estimate the values of $\frac{V}{K_3}$ and $\frac{1}{b}$. However, the fact that the divided-dose modality of administration evokes less response than the 1-minute modality can be interpreted to mean that several minutes after the infusion a significant fraction of the estrogen-receptor complex is still present in the cytoplasm. Using this concept and a trial-and-error method, we estimate that values for $\frac{V}{K_3}$ and $\frac{1}{b}$ are approximately 6 and 3 minutes, respectively. Substituting these in Equation 14A gives Equation 1 of the text:

$$(Q_N)_{tot} = C\,\epsilon_p[t + 3(1 - e^{-0.33t})].$$

The predicted time-course of β is shown in Fig. 6. Fig. 7 displays the time-course of Q_N. The relationship between calculated values of $\frac{bF(Q_N)_{tot}}{a\Phi K_2}$ and the flow response is depicted in Fig. 8. This figure shows that, regardless of the mode of administration, equal values of $(Q_N)_{tot}$ evoke the same blood flow response as required by the model. Furthermore, it shows that the relationship between $(Q_N)_{tot}$ and flow response is S-shaped. It should be noticed that the shape of the stimulus-response curve may depend on events subsequent to nuclear binding and that the model does not make any prediction concerning these events.

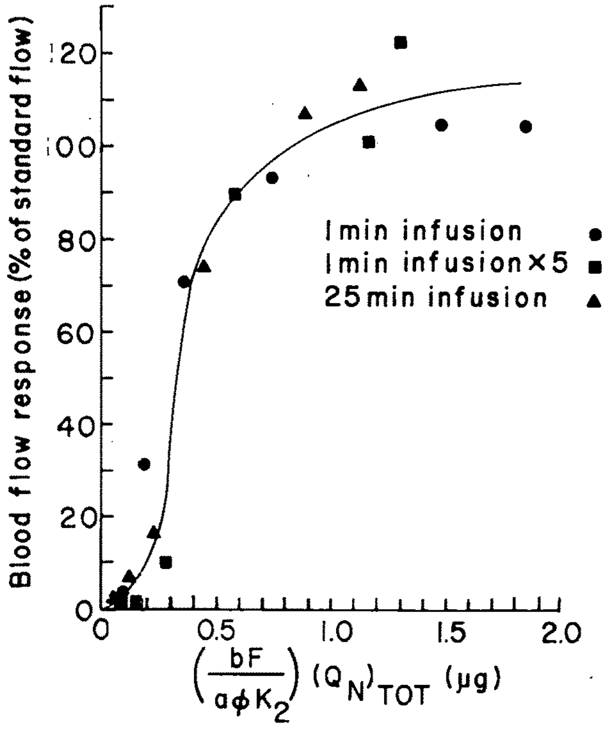

Fig. 8. Relationship of blood flow response to $(Q_N)_{tot}$. $(Q_N)_{tot}$ and the constants in $\frac{bF}{a\Phi K_2}$ were not measured in these experiments. The product $\left(\frac{bF}{a\Phi K_2}\right)(Q_N)_{tot}$ was estimated from the experimental data on the basis of a mathematical model. Since the quantity $\frac{bF}{a\Phi K_2}$ is a constant and $(Q_N)_{tot}$ is a variable, the dependence of the blood flow response on $(Q_N)_{tot}$ is as depicted.

Relationship of obstetric parameters to the concentration of pregnancy-associated plasma protein A

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Pregnancy-associated plasma protein A (PAPP-A) was measured by rocket immunoelectrophoresis in 272 patients at 34 weeks of pregnancy and within 48 hours before delivery. The values obtained have been compared to maternal parameters (age, weight, weight gain, blood group, rhesus factor, and parity) and to fetal parameters (sex, weight, Apgar score, rhesus factor, blood group, and placental weight). Maternal age, increased parity, and increased body weight are related to decreased PAPP-A levels. On the other hand, mothers carrying male fetuses, rhesus-negative children, and babies with Apgar scores higher than 7 at 1 minute have increased PAPP-A concentrations. These findings are discussed in relation to PAPP-A's involvement in the maternal immunologic system. (AM. J. OBSTET. GYNECOL. 138:494, 1980.)

PREGNANCY-ASSOCIATED PLASMA PROTEIN A (PAPP-A) is one of a growing list of newly discovered placental proteins. It was first partially purified from pregnancy plasma by Lin and associates.[1] With the help of an antiserum given to us by Dr. Lin, we purified PAPP-A to homogeneity and characterized it.[2, 3] PAPP-A is a macromolecular glycoprotein of about 800,000 molecular weight. Its concentration in maternal plasma increases as pregnancy progresses to reach a maximum just at parturition.[4, 5] After parturition it is cleared from the maternal circulation with a half life of 2 to 3 days.[4, 5]

The biological function of PAPP-A is as yet unknown but preliminary in vitro studies indicate that this protein might be involved in the complement system and in the fibrinolytic process.[3] The development of an immunoelectrophoretic technique for measuring PAPP-A[6] makes possible a different approach to the understanding of the physiologic role of PAPP-A. It was hoped that by studying the relationship between the concentrations of PAPP-A and some obstetric parameters a clue might be found regarding the function of PAPP-A.

Material and methods

Venous blood (10 ml) was drawn from randomly selected volunteers at the thirty-fourth week of pregnancy and in most cases within 48 hours of delivery when they were admitted in early labor. Plasma was separated and the samples were kept at −20° C until further processing.

Among the 272 patients with blood samples collected, 176 had normal pregnancies and 96 developed some obstetric abnormalities as judged by the case records obtained after delivery.

PAPP-A was measured by rocket immunoelectrophoresis[6] with a monospecific antiserum raised in rabbits against pure PAPP-A isolated from pooled third-trimester pregnancy plasma.[2] The standard was a pool of pregnancy plasma calibrated against pure PAPP-A.

As mentioned elsewhere,[7, 8] the PAPP-A concentration has a skewed distribution. By using a log transform of the data, the distribution is normalized and the t test can be applied.

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Received for publication May 8, 1980.

Accepted July 16, 1980.

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**Supported by a grant from the Royal Society.*

***Supported by a grant from the Medical Research Council.*

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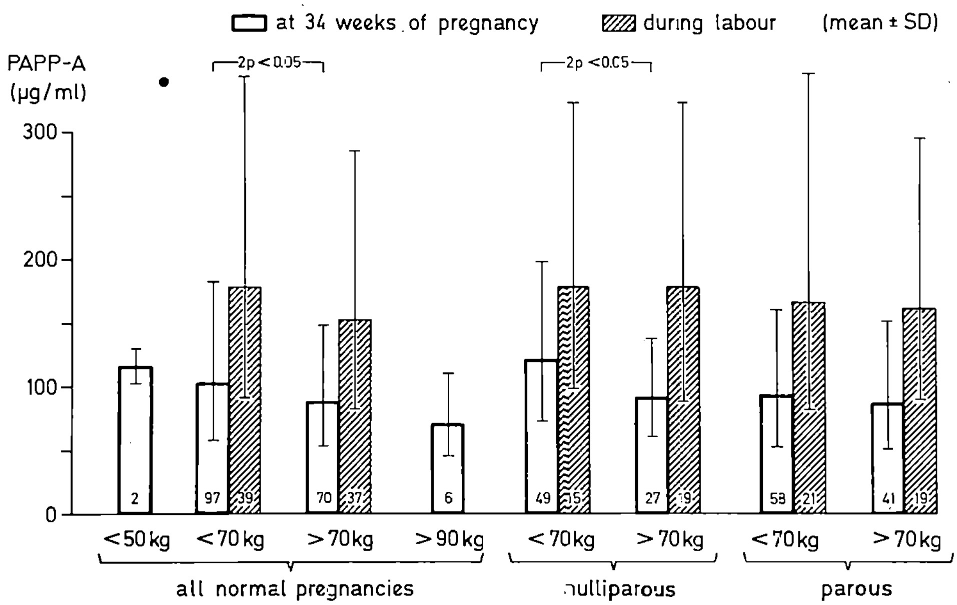

Fig. 1. Relation between maternal weight and PAPP-A concentrations (mean ± SD) at 34 weeks of pregnancy (open bars) and during labor (shaded bars).

Table I. Relation between maternal weight gain, baby weight, Apgar score, and PAPP-A concentrations

	PAPP-A at 34 weeks (mg/ml)					*PAPP-A during labor (mg/ml)*				
	n	*Mean*	*Mean + SD*	*Mean − SD*	*2P*	*n*	*Mean*	*Mean + SD*	*Mean − SD*	*2P*
Weight gain between 0 to 34 weeks:										
≥8 kg	84	92.6	160.5	53.5	NS	38	150.0	290.5	77.5	NS
<8 kg	64	102.8	184.9	57.1		30	185.1	350.2	97.8	
Normal baby weight	227	104.0	191.0	56.6	NS	99	167.8	328.6	85.6	NS
Babies below tenth percentile	31	110.8	178.9	68.6		16	199.6	343.7	115.9	
Apgar score at 1 min:										
≥7	158	95.9	167.0	55.1	NS	67	155.3	291.2	82.8	0.05
<7	18	107.5	170.3	67.8		10	243.4	454.3	130.4	

NS = Not significant.

Results

Maternal weight. The 34-week PAPP-A concentration in normal patients is inversely related to the maternal body weight (Fig. 1). Women under 50 kg have about twice the concentration of PAPP-A of those over 90 kg. When the PAPP-A levels during labor are considered, the same significant difference is observed and in the 34-week group there is a steady fall in PAPP-A concentration as maternal weight increases.

When the patients are divided into parous and nulliparous women, the PAPP-A levels at 34 weeks are significantly lower for nulliparous women over 70 kg as compared to women weighing less than 70 kg.

Maternal age. PAPP-A concentrations at 34 weeks show a tendency to decline with maternal age. This becomes statistically significant only if the patients less than 30 are compared with those patients older than 35 years of age. When estimates are done within 48 hours of delivery, a clear-cut significant decrease is observed (Fig. 2).

When the age effect is tested separately in nulliparous and parous women, the observed decrease is not significant anymore. It is difficult to separate the effects of parity and of age on PAPP-A concentration, but on the whole it appears that parity effects are dominant.

Parity. It is problematic whether a previous abortion should count as a pregnancy in the parity categorization of our data. If all the patients with a history of previous abortion are eliminated, there remain 138 normal women with 34-week PAPP-A values. These subjects show a consistent decline in PAPP-A with increasing parity, as do the 64 patients "in labor" (Fig. 3).

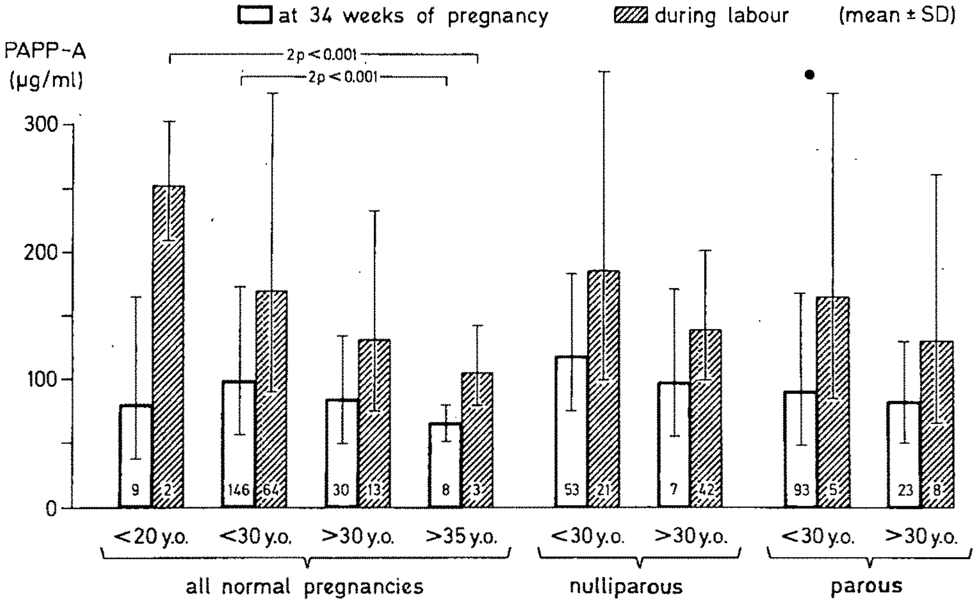

Fig. 2. Relation between maternal age and PAPP-A concentrations (mean ± SD) at 34 weeks of pregnancy (open bars) and during labor (shaded bars).

Table II. Correlation between placental weight and PAPP-A concentrations in normal pregnancies

	Spearman rank correlation coefficients			
	34 weeks		*Labor*	
	PAPP-A	*Log PAPP-A*	*PAPP-A*	*Log PAPP-A*
Coefficient	−0.104	−0.104	0.151	0.151
n	176	176	76	76
2P	0.18	0.18	0.19	0.19

As can be seen in Fig. 3, previous abortions do not of themselves make a difference to the PAPP-A concentration. Therefore, when the normal population is split into parous and nulliparous groups and the existence of a previous abortion is ignored, the parous group has a significantly lower PAPP-A concentration than the nulliparous group at 34 weeks.

Weight gain in pregnancy. The mean weight gain between 0 and 34 weeks of pregnancy in the normal patients was 7.9 ± 3.5 kg (mean ± SD).

No significant differences are observed when the PAPP-A levels of normal patients with a body weight gain of more than 8 kg are compared to those of patients with a weight gain of less than 8 kg (Table I).

Baby weight. When all pregnancies are considered, the mothers whose babies are growth retarded (below tenth percentile according to the criteria of Thomson and associates[12]) do not have significantly lower PAPP-A levels than those carrying normal-sized babies (Table I). Within the normal group there was no significant correlation (Spearman rank) between PAPP-A and baby weight.

Apgar score. As might be expected, there was no relationship between the PAPP-A concentrations at 34 weeks and the Apgar score at birth but Table I shows a significant tendency for low PAPP-A values in labor to be associated with lower Apgar scores.

Placental weight. Spearman rank correlation coefficients have been calculated in order to determine whether PAPP-A concentrations bear any relation to placental weight.

Among the 176 normal pregnancies no statistically significant correlation could be found between the placental weight and the concentrations of PAPP-A at 34 weeks or during labor (Table II).

These placental weights have been taken from the routine labor records. Although we have tried to maintain a standardized weighing technique, it must be acknowledged that many observers were involved and the weighing often was done when there was much pressure of other work. There may be some doubt about the accuracy of these data.

Fetal sex. At 34 weeks there is no effect of fetal sex on PAPP-A. Some of the subgroups in the patients "in labor" show significantly larger PAPP-A values in male babies. This effect disappears when mothers over 30 or parous mothers are considered (Fig. 4).

Rhesus factor. Unfortunately, not all mothers or babies were tested for rhesus factor, but when PAPP-A

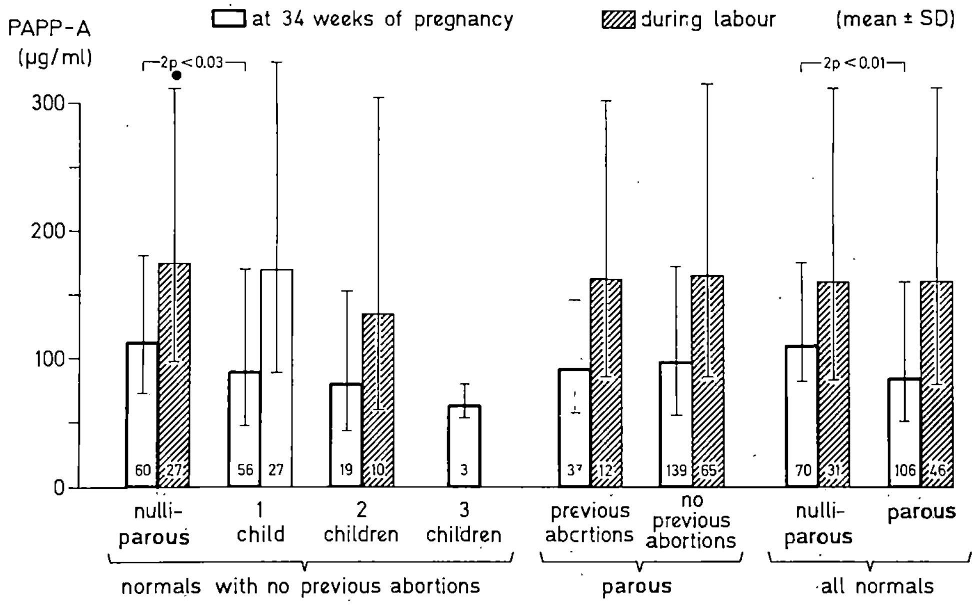

Fig. 3. Relation between parity and PAPP-A concentrations (mean ± SD) at 34 weeks of pregnancy (open bars) and during labor (shaded bars).

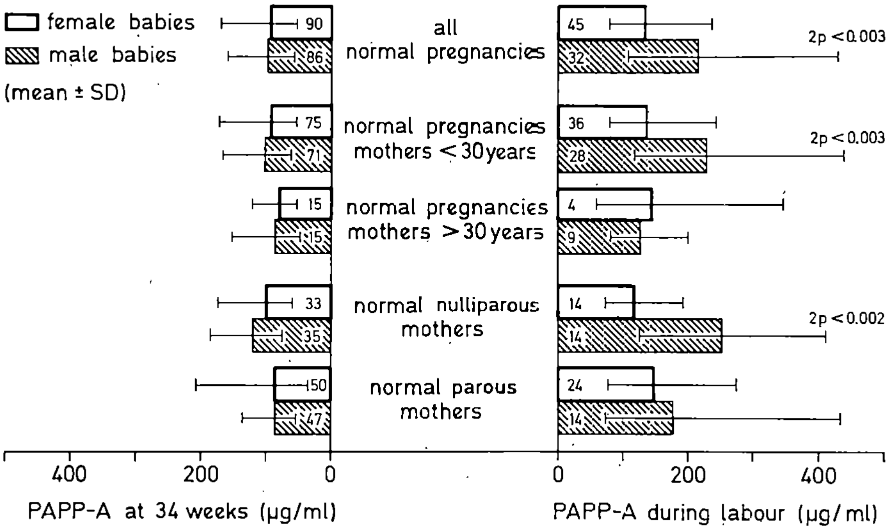

Fig. 4. Relation between fetal sex and PAPP-A concentrations (mean ± SD) in mothers with female babies (open bars) and in mothers with male babies.

levels of rhesus-positive and rhesus-negative mothers are compared no significant differences are observed (Fig. 5). However, mothers with rhesus-negative babies, irrespective of their own rhesus factor or of their parity, have significantly higher concentrations of PAPP-A as compared to mothers with rhesus-positive babies (Fig. 5).

Maternal and fetal blood groups. In the cases where the blood group was assessed, no changes in concentrations of PAPP-A at 34 weeks or during labor are observed when normal patients are divided according to their blood groups or according to the blood groups of their children (Table III).

Comment

The inverse relationship between PAPP-A and maternal body weight may be due to a dilution effect, the heavier women having a larger plasma volume. The reduced PAPP-A levels in women over 35 years of age could be due to the effect of parity, although when

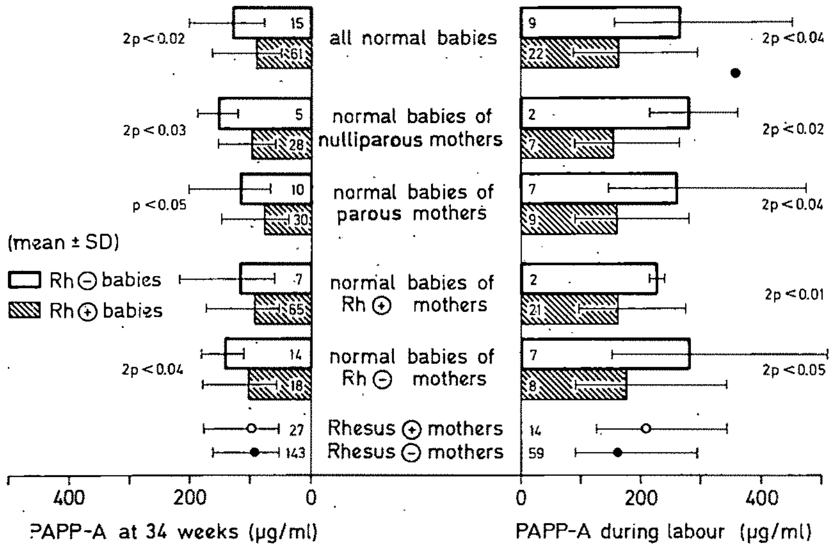

Fig. 5. Relation between rhesus factors and PAPP-A concentrations (mean ± SD).

Table III. Relation between maternal and infant blood group and PAPP-A concentrations

	PAPP-A at 34 weeks					*PAPP-A during labor*				
	n	*Mean*	*Mean + SD*	*Mean − SD*	*2P*	*n*	*Mean*	*Mean + SD*	*Mean − SD*	*2P*
Infants:										
A	23	95.0	198.6	45.5		8	194.0	293.7	128.2	NS
B	7	101.8	147.2	70.5	NS		–	–	–	–
O	39	97.3	157.7	60.0		20	177.0	329.5	95.0	NS
Mothers:										
A	51	95.0	173.0	52.2		21	173.0	337.2	88.7	
B	24	114.3	173.0	75.5	NS	8	181.1	307.6	106.6	NS
O	85	97.3	161.4	58.6		39	165.2	314.7	86.7	

parity is allowed for in the age analysis there is a nonsignificant tendency for older women to have a lower PAPP-A concentration.

The clear-cut relationship of parity and PAPP-A concentration is intriguing. This is an effect which is triggered by some event in late pregnancy since an abortion does not lower PAPP-A in a subsequent pregnancy. If PAPP-A is a placental product, then the concentration of this protein is presumably determined primarily by fetoplacental factors. This is borne out by the likely connection between PAPP-A values and the fetal rhesus factor. However, the "information" of a previous pregnancy can only be stored in the maternal organism and the mother must have some means of conveying this to the fetoplacental unit. A likely mechanism is an immunologic factor. It may be that immunologic factors in the mother, triggered by pregnancy, affect PAPP-A production by the fetoplacental unit in a subsequent pregnancy.

A further indication that the PAPP-A production rate might be controlled to some extent by maternal factors arises from our finding that, in contrast to the conclusions reached by Lin and associates,[9] there is no clear-cut relationship between PAPP-A concentration and placental weight (with the relative inaccuracy of our data on placental weights taken into consideration). It has been convincingly argued that the placenta is an independent organism, independent of feedback mechanisms whose production rate of secreted proteins is solely related to placental size and intervillous blood flow.[10] It must be acknowledged that the evidence that PAPP-A is a protein produced by the placenta rests largely on one publication which demonstrates its presence in the syncytiotrophoblast by immunofluorescence staining.[11]

If PAPP-A is actually a maternal product it seems reasonable that maternal rather than fetoplacental factors should influence its concentration in maternal serum.

Our thanks are due to Mrs. P. Cunningham for technical assistance.

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Steroid concentrations and $\Delta^5,3\beta$-hydroxysteroid dehydrogenase activity in human placenta

Comparison between elective cesarean section and spontaneous vaginal delivery

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The level of $\Delta^5,3\beta$-hydroxysteroid dehydrogenase ($\Delta^5,3\beta$-HSDH) was measured in the mitochondrial and microsomal fractions of the human term placenta. The capability of the placenta to transform pregnenolone to progesterone was found to be significantly higher after spontaneous vaginal delivery than after elective cesarean section ($p < 0.001$). There was a significant rise in the placental concentration of estrone occurring at the time of parturition ($p < 0.01$). The placental concentration of estradiol-17β was also greater after than before parturition but the difference was not statistically significant. Conversely to the estrogen concentration, no difference existed in the progesterone concentration between the two groups of placentas. The significance of these findings is discussed in relation to the parturition process. (AM. J. OBSTET. GYNECOL. 138:500, 1980.)

THE MECHANISM that triggers parturition is not as well understood in the human as in the "sheep model."[1, 2] Ethical and practical problems limit the possibility to determine to what extent the human fetus has a decisive role in the timing of labor. In addition, near term, numerous authors have failed to detect the significant fall in progesterone and rise in estrogens in the maternal peripheral plasma which are considered as prerequisites of parturition, according to the concept of Csapo.[3] This fact could be partly explained by the original distribution of the enzymes responsible for both the synthesis and the metabolism of steroid hormones in the different tissues of the human fetoplacental unit. However, the changes that occur in placental steroidogenesis before the onset of labor should be considered. An essential enzyme for the formation of progesterone and estrogens is $\Delta^5,3\beta$-hydroxysteroid dehydrogenase ($\Delta^5,3\beta$-HSDH; EC 1.1.1.51) which catalyzes the rate-limiting step in the conversion of pregnenolone to progesterone and of dehydroepiandrosterone to androstenedione. Previous studies performed on human term placentas have shown that the enzymatic activity is mainly localized in mitochondrial and microsomal fractions.[4, 5]

In view of these findings, we have attempted to study the relationship between human placental $\Delta^5,3\beta$-HSDH and parturition. Endogenous levels of progesterone, estradiol-17β, and estrone were also determined for comparison.

Material and methods

Placentas were collected immediately, either at the time of elective cesarean section performed prior to beginning of labor after the thirty-ninth week of gestation or after spontaneous vaginal delivery at term. All women were in good health with uncomplicated preg-

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This work was supported by I.N.S.E.R.M. (ATP No. 79.107).

Received for publication January 4, 1980.

Revised March 24, 1980.

Accepted July 17, 1980.

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Table I. Δ^5,3β-HSDH activity in human placenta in relation to parturition

Mode of delivery	*Δ⁵, 3β-HSDH activity (nmoles/mg protein/min)**	
	Mitochondrial fraction	*Microsomal fraction*
Cesarean section	63 ± 40 (15-130) n = 10	53 ± 30 (19-100) n = 8
Vaginal delivery	191 ± 48 (101-260) n = 15	253 ± 36 (190-285) n = 8
Significance	$p < 0.001$	$p < 0.001$

*Activity is expressed as the mean ± SD and extreme values.
n = Number of placentas assayed.

Table II. Steroid concentrations in human placenta in relation to parturition

Mode of delivery	*Steroid concentrations (ng/gm wet weight tissue)**		
	Progesterone	*Estradiol-17β*	*Estrone*
Cesarean section	3,128 ± 1,045 (1,916-4,600)	13 ± 10 (4-29)	176 ± 66 (90-270)
Vaginal delivery	2,847 ± 1,028 (1,545-4,061)	45 ± 36 (18-100)	410 ± 90 (329-545)
Significance	$p > 0.1$	$p < 0.1$	$p < 0.01$

*Steroid values are expressed as the means ± SDs and extreme values of duplicate experiments in five different placentas.

nancies, the pregnancy being terminated by cesarean section because of cephalopelvic disproportion. Dissection and homogenization procedures and all the further steps required to obtain mitochondrial and microsomal fractions were described elsewhere.[5]

Aliquots (0.5 ml) of placental homogenate (200 mg of wet dissected tissue per 6 ml of distilled water) were treated three times by 2 ml of hexane for progesterone extraction and twice by 5 ml of ether:ethyl acetate (3:1, v:v) for estradiol-17β and estrone extraction. The endogenous content in these three steroids was then determined by means of a direct radioimmunoassay, as described previously.[6]

Δ^5,3β-HSDH activity was measured in mitochondrial and microsomal fractions without delay following their preparation, according to a double-isotope dilution method in conditions similar to those reported earlier.[5] Briefly, 1 ml of the enzymatic preparation was added to 9 ml of the incubation medium in such a manner that the final concentrations of components in the reaction mixture were: 5×10^{-5}M [16-^{3}H]pregnenolone (5×10^{-2} μCi), 5×10^{-4}M NAD$^+$, 1×10^{-2}M NaCl, 1.5×10^{-2}M KCl, 2×10^{-2}M sucrose, 2×10^{-3}M tris HCl, pH 7.4. At various times 1 ml of the incubation medium was pipetted. The enzymatic reaction was stopped by immediate extraction with ether:ethyl acetate (1:1, v:v). At the same time, 5×10^{-3} μCi of [4-^{14}C]progesterone was added as a tracer. Thin-layer chromatography in the chloroform acetone (185:15, v:v) system was used to separate progesterone (Rf 0.69) from pregnenolone (Rf 0.48).

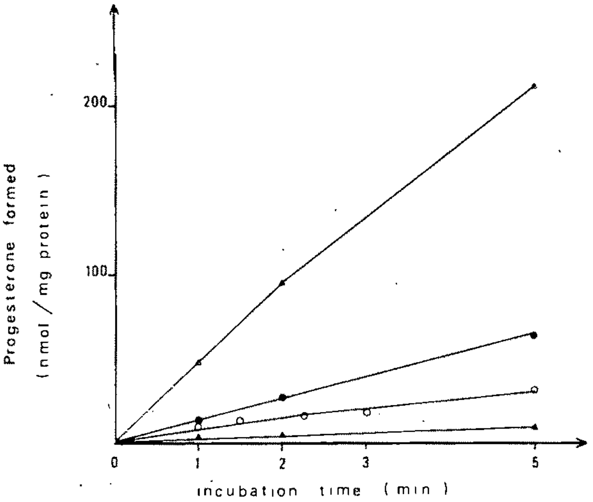

Fig. 1. Kinetics of conversion of pregnenolone to progesterone by mitochondrial fraction from human placenta obtained by elective cesarean section. The concentrations of cofactor used are 1×10^{-4}M NAD$^+$ (△—△), 1×10^{-4}M NADP$^+$ (●—●), 1×10^{-4}M NADPH (▲—▲), none (○—○).

Protein concentration in each fraction was measured by the method of Lowry and associates[7] with bovine serum albumin used as a standard.

Student's t test was used for determination of the statistical significance of differences between mean values.

Results

The conversion rate of pregnenolone to progesterone by the mitochondrial fraction of a placenta obtained by elective cesarean section is illustrated in Fig. 1. It was found that NAD$^+$, rather than NADP$^+$, is the required cofactor. However, the mitochondrial fraction is able to transform pregnenolone to progesterone without any addition of exogenous cofactor. This conversion, which is not as high as that in the presence of NAD$^+$ or NADP$^+$, is appreciably reduced by the addition of NADPH. Although experiments were performed at subsaturating substrate concentrations and nonlimiting cofactor concentrations, it should be noticed that the amount of progesterone formed does not vary linearly with time after about 3 to 5 minutes. The decrease of the rate of pregnenolone oxidation can be attributed to an inhibitory effect of the steroid product, as previously observed.[5] In order to calculate specific activities, conditions were chosen so that the progesterone formed by the mitochondrial and microsomal enzymes was linearly dependent on the time of incubation and on the protein content. Specific activities determined in relation to the parturition process are reported in Table I. It can be seen that the Δ^5,3β-HSDH activity is significantly higher ($p < 0.001$) in the placentas obtained after vaginal delivery than in the organs obtained during cesarean section. A similar trend may be observed in both subcellular fractions, although the extent of the increase appears somewhat higher in the microsomal fraction than in the mitochondrial one.

The concentrations of progesterone, estradiol-17β, and estrone in placentas taken during cesarean section or after vaginal delivery are shown in Table II. There is no significant difference in the concentration of progesterone for the placentas obtained either by cesarean section or after vaginal delivery. Conversely to progesterone, changes in estrogen values could be discerned after parturition. The mean estrogen levels in placentas obtained after vaginal delivery are greater than in organs obtained during cesarean section. However, the difference is not statistically as significant for estradiol-17β ($p < 0.1$) as for estrone ($p < 0.01$). In the two groups of placentas, the mean estrone level is always about tenfold higher than the estradiol-17β value.

Comment

The present study shows that the human placenta possesses a higher capacity to synthesize progesterone from pregnenolone after parturition than before. In the umbilical plasma as well as in the liquid of perfusion of the whole placenta, previous investigations have revealed that the pregnenolone:progesterone ratio was more elevated after elective cesarean section than after spontaneous labor with vaginal delivery.[8] This may be related to the increase of placental Δ^5,3β-HSDH activity throughout parturition. Presumably, different experimental conditions, including the use of solubilized mitochondrial enzyme, might account for the lack of significant differences reported by Diaz-Zagoya and associates[9] for Δ^5,3β-HSDH activity between placentas obtained by elective cesarean section and those obtained after spontaneous vaginal delivery. Indeed, placental Δ^5,3β-HSDH activity seems to be intimately associated with mitochondrial and microsomal membranes,[5] and the instability of the enzyme toward solubilization is well known.[10] Thus, as for several other membrane-bound enzymes, the possibility exists that the transfer of activity to a soluble state is followed by the loss of "induced" effects. The reason for the increased specific activity of the Δ^5,3β-HSDH in the placentas obtained after parturition remains obscure. It should be mentioned that in the human fetal adrenals the conversion of pregnenolone to progesterone is not favored, an endogenous inhibitor probably being implicated in this relative deficiency of a pregnenolone-specific Δ^5,3β-HSDH–isomerase system.[11] In the human placenta, it is well established that various factors like reduced forms of cofactors and a number of steroids are able to affect the "in vitro" Δ^5,3β-HSDH activity. Obviously, some of them change with parturition. Of interest in this regard is the recent characterization by Wiener and Reiner[12] of a nonpolar inhibitor, common for placental Δ^5,3β-HSDH and 20α-hydroxysteroid oxidoreductase enzymes, which is present in both the soluble and the membranous fractions. It has been suggested by Wiener[13] that this inhibitor is involved with partial suppression of placental Δ^5,3β-HSDH activity during normally progressing gestation. Release of this inhibitor occurs in situations involving placental damage such as x-irradiation.[14] Therefore, this finding opens up the possibility that the high Δ^5, 3β-HSDH level found in placentas obtained by vaginal delivery may be due to the placental damage produced by hypoxia and ischemia during parturition. However, we do not have enough information at our disposal to establish the physiologic significance of increased activity after parturition. Moreover, if one considers that the human placenta is often described as a senescent organ at term, these data appear rather surprising.

Our results for progesterone concentration agree closely with those reported by other authors in human placenta, near term.[6, 9, 15, 16] Nevertheless, our data obtained with Δ^5,3β-HSDH do not correlate with the endogenous progesterone content, which was found to be similar in the two groups of placentas. In any case, these results do not indicate that the "progesterone withdrawal," which is considered as a necessary event in the initiation of labor, takes place in the placenta. Since there is a marked increase in the conversion of progesterone into 20α-dihydroprogesterone from midpregnancy until term and during parturition,[9, 17] it is possible that 20α-hydroxysteroid oxidoreductase contributes to the maintenance of a constant progesterone level in the placenta. In spite of the expected fall in progesterone, we observed a rise in the placental estrogen content with parturition. Estrogens are known to cause lability of the lysosomal membrane and promote prostaglandin synthesis, and it was also demonstrated that placental estrogen production is stimulated by prostaglandins.[18] Consequently, the increased estrogen content observed in the placenta may be related to the increased placental prostaglandin synthesis occurring with parturition. Previous works have demonstrated that placental steroids are able to impregnate strongly the adjacent fetal membranes and uterine tissues.[6, 15, 16, 19] However, it should be remembered that the level of steroids in these structures is also controlled by binding capacity and metabolizing enzymes. However, near term, estrogens, especially estradiol-17β, appear to diffuse more easily than progesterone from the placenta to the adjacent myometrial area.[6] It is likely that the shift from progesterone to estrogen dominance observed in the placenta at parturition contributes to modification of the myometrial estrogen:progesterone ratio. Unfortunately, we have no data to prove whether the variation observed is involved in the onset of labor or if it is the result of labor. Nevertheless, our results are compatible with the concept that local change in the steroidal environment increases the myometrial excitability at the time of parturition.

We wish to thank Dr. M. Bedin for revising the text.

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Postpartum lymphocytic thyroiditis in American women: A spectrum of thyroid dysfunction

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Six episodes of primary hypothyroidism followed pregnancy in five women. Within 6 months post partum, all were clinically hypothyroid, with a diffuse goiter of two to three times normal size, altered thyroid function tests (average thyroxine [T_4] = 2.6 μg/dl, thyroid-stimulating hormone [TSH] = 58.2 μU/ml), and elevated antithyroid antibodies. (All had antimicrosomal antibody titers of 1:6,400 or more.) After 5 more months, all patients had a decrease in antibody titer, resolution of symptoms, and recovery of thyroid hormone levels (average T_4 = 7.3 μg/dl), although two patients had compensated hypothyroidism (their average TSH = 9.3 μU/ml). Four patients had lymphocytic thyroiditis diagnosed by fine-needle aspiration biopsy. Despite the apparent transience of postpartum hypothyroidism, all five patients have chronic thyroid dysfunction: three women have persistent goiters (all less than twice normal size) and two have compensated hypothyroidism; one patient had previous Graves' hyperthyroidism, and two had recurrent transient hypothyroidism (at 21 months postpartum in one patient, and 5 months after another pregnancy in the other). Three women had transient, painless hyperthyroidism (average T_4 = 16.1) before becoming hypothyroid: two patients within 8 weeks post partum, and the third patient at 20 months (preceding an episode of recurrent hypothyroidism). Previous reports of postpartum hypothyroidism have come from scattered locales abroad, and it is unclear whether local environmental factors are pathogenic. Nevertheless, this syndrome may, in general, have gone unrecognized and may be a significant cause of illness in postpartum women. (AM. J. OBSTET. GYNECOL. 138:504, 1980.)

CHRONIC lymphocytic thyroiditis and Graves' disease are now recognized as autoimmune disorders.[1] Recently, a new syndrome has been described that resembles that of subacute thyroiditis in its clinical presentation but without neck pain. This syndrome is accompanied by positive thyroid autoantibodies and biopsy-proved lymphocytic thyroiditis in some cases.[2] It has been given various names, including "painless" or "silent" thyroiditis. Whether this represents a chronic form of granulomatous thyroiditis or an acute form of lymphocytic thyroiditis has been debated. A similar controversy exists over the recently described syndrome of postpartum thyroiditis. This entity, initially reported in Japanese women[3] and recently described in England,[4] has been characterized by transient hypothyroidism, goiter, and antithyroid autoantibodies occurring within 6 months of delivery and remitting by 1 year. Five other patients, reported from Canada,[5] had transient hyperthyroidism shortly after pregnancy, two of whom subsequently became hypothyroid.

We now report five new cases of postpartum thyroiditis in American women. In contrast to the previous reports, which have stressed the transient nature of all these forms of thyroiditis,[3-5] our study of five patients revealed evidence of persistent thyroid disease in all of them. In addition, our patients had high titer antithyroid antibodies and aspiration biopsies showing lymphocytic thyroiditis, which suggests the presence of an autoimmune disorder. Despite this evidence for an underlying chronic disorder, the transience of the

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Received for publication December 13, 1979.

Revised March 4, 1980.

Accepted March 27, 1980.

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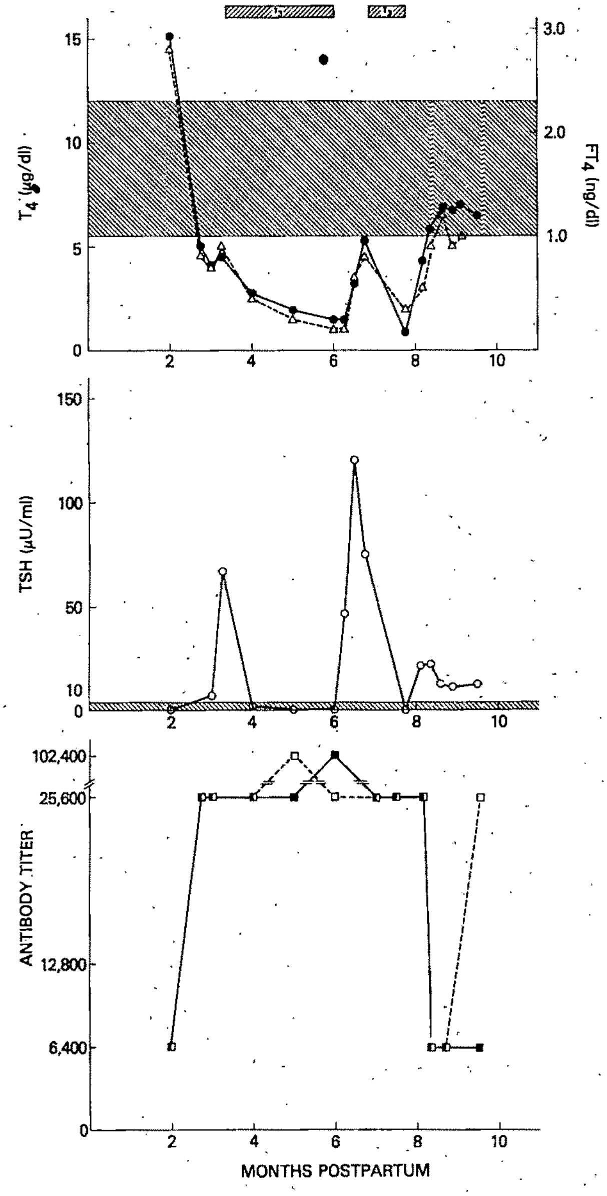

Fig. 1. Thyroid function tests and antibody levels after delivery in Case 1. Serum T_4 (●——●) and free T_4 (△----△) are shown in the top panel. Serum T_3 levels (not shown) paralleled serum T_4, except when the patient was treated with L-triiodothyronine (T_3) as indicated in the upper panel. During those periods, serum T_3 was always normal. TSH levels (○——○) are shown in the middle panel, and antimicrosomal antibody (■——■) and antihyroglobulin (□——□) titers in the lower panel. Normal values are within the shaded areas.

symptoms of hypothyroidism may result in many cases going unrecognized both by the patients themselves and by their physicians.

Methods

Subjects. All patients were evaluated and treated at the National Institutes of Health (NIH) outpatient clinic. Cases 1 and 2 were first noticed by the husbands, both NIH physicians, when goiter appeared post partum; Case 3 was referred for the same reason by the NIH arthritis clinic. Cases 4 and 5 were being followed at NIH for prior thyroid disease.

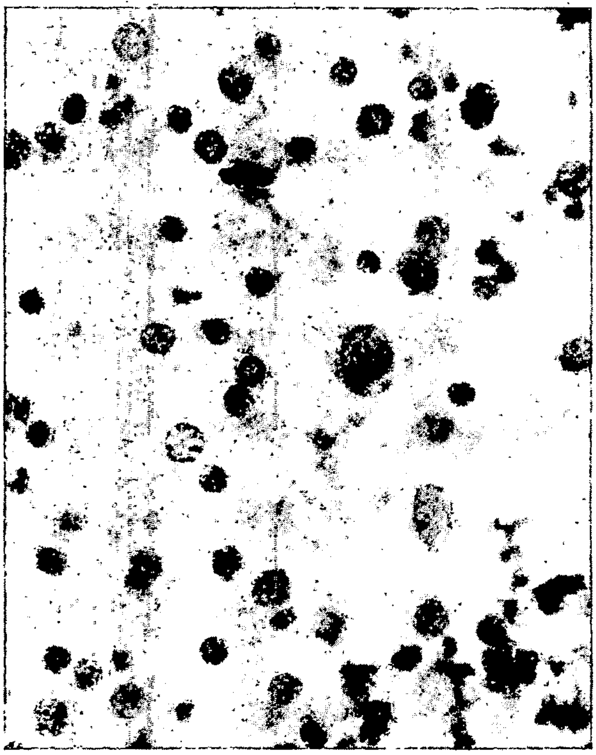

Fig. 2. Filter prepared from thyroid aspirate of Case 1 demonstrates many small and medium-sized lymphocytes and plasma cells. A germinal center cell, with prominent nucleolus and cytoplasm, is in the center of the photomicrograph. A diagnosis of lymphocytic thyroiditis was made. (Original magnification, ×500.)

Assays. Serum thyroxine (T_4), triiodothyronine (T_3), and thyroid-stimulating hormone (TSH) were measured by standard radioimmunoassay techniques. T_3 resin uptake (T_3RU) was measured by a standard technique, and free thyroxine (FT_4) was measured by a dialysis method. Unless otherwise stated, normal values are: T_4, 5.5 to 11.5 μg/dl; FT_4, 1.0 to 2.3 ng/dl; T_3RU, 22 to 35%; T_3, 54 to 218 ng/dl; and TSH, less than 3.9 μU/ml. Antithyroglobulin and antithyroid microsomal antibodies were measured by Bio Science Laboratories with the use of hemagglutination titers for all patients except Case 3, whose antimicrosomal antibodies were measured by Nichols Institute by competitive binding radioassay.

Case reports

Case 1. A 27-year-old white woman gave birth in September, 1978, after her first pregnancy. She had no

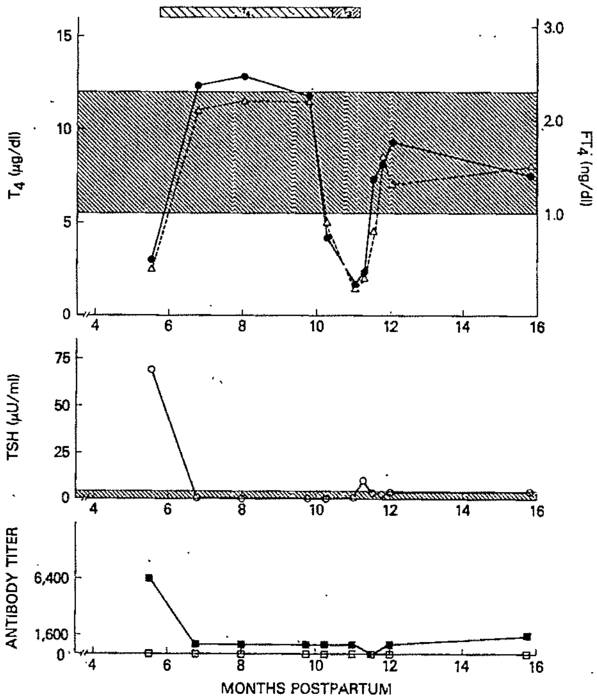

Fig. 3. Thyroid function tests and antibody levels after delivery in Case 2. Legend as in Fig. 1. L-Thyroxine (T_4) and triiodothyronine (T_3) were administered as indicated in the upper panel.

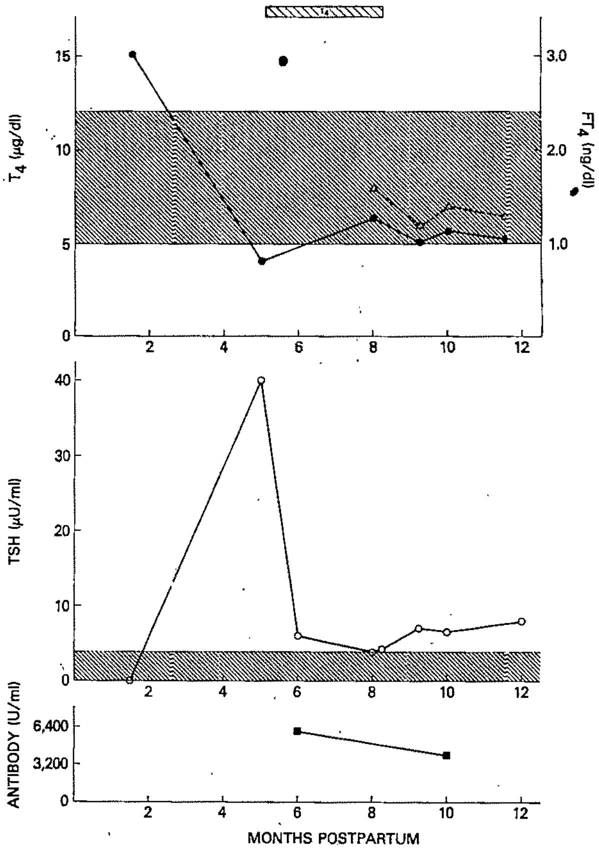

Fig. 4. Thyroid function tests and antibody levels after the third delivery in Case 3. Legend as in Fig. 1.

history of thyroid disease until her obstetrician noticed a small goiter during the third trimester; serum T_4 was 9.9 μg/dl (normal, 3.9 to 14.7). The goiter gradually enlarged after delivery. When examined 2 months post partum, she was asymptomatic, with a painless, firm thyroid of twice normal size. Thyroid function tests at that time were elevated: T_4, 13.3 μg/dl; FT_4, 2.9 ng/dl; T_3, 280 ng/dl; and TSH, less than 1.0 μU/ml. Antithyroglobulin and antimicrosomal antibody titers were also elevated (Fig. 1). Since the patient was nursing her infant, no ^{131}I uptake test was performed. No therapy was given, and within 6 weeks the patient felt sluggish and easily fatigued. Her thyroid was then found to be firm and diffusely enlarged to four times normal size. She was hypothyroid: T_4, 4.3 μg/dl: FT_4, 0.8 ng/dl; T_3, 106 ng/dl; and TSH, 7.6 μU/ml. Fine-needle thyroid aspiration biopsy[6] performed at that time revealed numerous lymphocytes, plasma cells, histiocytes, a few polymorphonuclear leukocytes, and germinal center cells; a diagnosis of lymphocytic thyroiditis was made (Fig. 2). Administration of L-triiodothyronine, 25 μg three times a day, was begun, with prompt resolution of symptoms. By 7 months post partum the thyroid was moderately soft at upper normal size. T_3 was then withheld, and 3 weeks later the goiter had enlarged to three times normal size. Thyroid function tests were: T_4, 5.3 μg/dl; FT_4, 0.8 ng/dl; T_3, 173 ng/dl; and TSH, 56.1 μU/ml. The patient felt sluggish and fatigued. The administration of triiodothyronine was then resumed for 1 month, during which time the thyroid regressed to upper normal size. T_3 was again withheld, and thyroid function tests at 8½ months post partum were: T_4, 4.9 μg/dl; FT_4, 1.0 ng/dl; T_3, 192 ng/dl; and TSH, 11.3 μU/ml. The patient has felt well, but the goiter has remained moderately firm and diffusely enlarged to 1½ times normal size. Thyroid function tests 9½ months post partum were unchanged from the previous month. Family history reveals no endocrine or autoimmune disease. The infant has grown and developed normally, without thyroid dysfunction.

Case 2. A 28-year-old white woman gave birth in February, 1978, after her first pregnancy. She was asymptomatic, with no previous history of thyroid disease until she noticed gradually increasing fatigue and a visible goiter 4½ months post partum. Examination revealed a firm, painless thyroid 2½ times normal size, with: T_4, 2.0 μg/dl; FT_4, 0.5 ng/dl; T_3, 84 ng/dl; and TSH, 58.5 μU/ml. The antimicrosomal antibody titers were elevated (Fig. 3). Twenty-four-hour ^{131}I uptake was 30%; scanning revealed diffuse homogeneous uptake, with visualization of a pyramidal lobe. Prednisone, 10 mg four times a day for 1 week, was given to shrink the thyroid.[7] This resulted in a 30% decrease in the size of the goiter. Administration of L-thyroxine, 0.2 mg daily, was then begun. Symptoms resolved within 3 weeks, and by 10 months post partum, the thyroid was barely palpable. Aspiration biopsy at that time revealed many lymphocytes, histiocytes, and occasional germinal center cells; a diagnosis of lymphocytic thyroiditis was made. Therapy was then changed to L-triiodothyronine, 25 μg three times a day, which was discontinued 11 months post partum. The patient continued to feel well, and within 1 more month the thyroid function tests were normal. The TSH response to thyrotropin-releasing hormone (TRH) was also normal, rising from a basal 3.9 μU/ml to 25.4 μU/ml at 30 minutes. The thyroid was moderately firm and slightly larger than normal. The patient has remained euthyroid for 6 months, but the small goiter persists. Family history includes rheumatoid arthritis in the patient's mother, but no endocrine disease. The infant has grown and developed normally, without thyroid dysfunction.

Case 3. A 29-year-old white woman gave birth in September, 1972, after her first pregnancy. She had no known signs or symptoms of thyroid disease until 3 months post partum, when she became fatigued and noticed a visible, painless goiter. One month later, thyroid function tests showed: T_4, 1.5 μg/dl (normal 4.5 to 10.7) and T_3RU, 24%. Twenty-four-hour ^{131}I uptake was 21%, with a homogeneous, moderately enlarged thyroid on scan. Treatment was begun with L-thyroxine, 0.1 mg daily. The patient discontinued this 2 months later, without any further fatigue, and the goiter disappeared within 6 months without therapy. In October, 1974, she had visual and auditory hallucinations and arthralgias, but no rash or fever. Testing for antinuclear antibody was positive. The diagnosis of systemic lupus erythematosus was made, and prednisone, 20 mg daily, was begun, with prompt resolution of symptoms. She gave birth again in February, 1976, remaining on prednisone throughout her pregnancy and postpartum period in doses of 20 to 60 mg daily. She had no clinical thyroid dysfunction, and no thyroid function tests were performed during or after this pregnancy. In September, 1976, she was referred to the NIH, where an extensive serologic examination was negative for systemic lupus. The prednisone was tapered and discontinued in June, 1977, without complications.

The patient gave birth again in May, 1978. Six weeks post partum, she noticed trembling, headache, insomnia, and increased appetite. She had no neck pain. Thyroid hormones were elevated (T_4, 15μg/dl; T_3RU, 37%), and 24-hour ^{131}I uptake was 6%. The symptoms cleared within 3 weeks, only to be followed 2 months later by clinical and chemical hypothyroidism: T_4, 4.3 μg/dl; TSH, 40 μU/ml (Fig. 4). Elevated antimicrosomal antibodies were found, and L-thyroxine, 50 μg daily, was begun. Aspiration biopsy in January, 1979, revealed lymphocytes, histiocytes, plasma cells, and germinal center cells; a diagnosis of lymphocytic thyroiditis was made. The thyroxine was then withheld, and the patient remained asymptomatic. Since then, she has had a barely palpable, moderately firm thyroid, without nodularity. Thyroid hormones have remained normal through 14 months post partum (T_4, 5.2 μg/dl; FT_4, 1.3 ng/dl; T_3, 165 ng/dl). TSH is 7.9 μU/ml, and hyperresponds to TRH (37.2 at 30 minutes). Family history reveals idiopathic hypothyroidism in her mother and a brother, but no other endocrine or autoimmune disease. All children have been euthyroid, and have grown and developed normally.

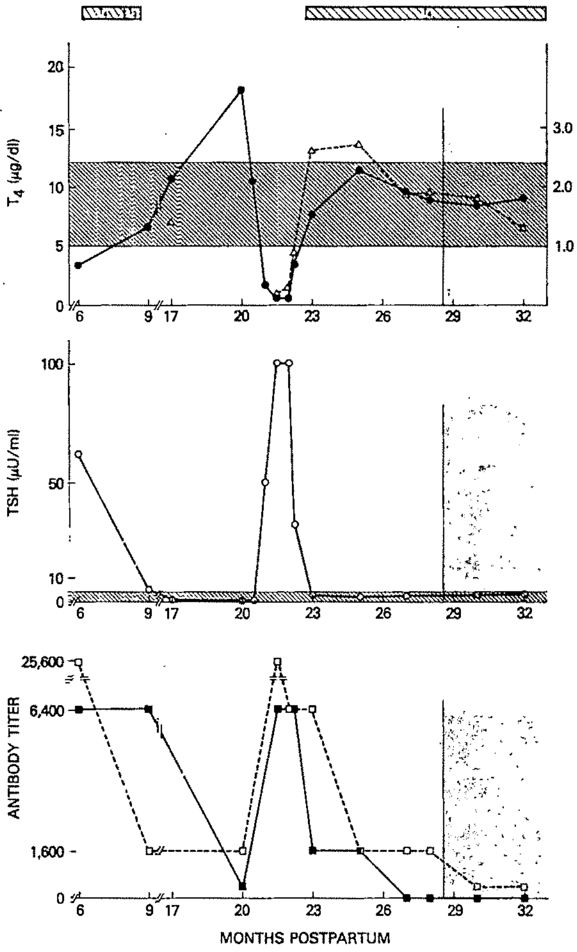

Fig. 5. Thyroid function tests and antibody levels after the second delivery in Case 4. Legend as in Fig. 1. The patient is currently pregnant, as indicated by the stippled areas.

Case 4. A 26-year-old white woman gave birth to her second child in July, 1976. She was well until she noticed a goiter, increased weight, and fatigue 6 months later. There was no previous history of thyroid disease. She was hypothyroid (T_4, 3.4 μg/dl; T_3, 75 ng/dl [normal, 80 to 160]; TSH, 62 μU/ml), with elevated antithyroglobulin and antimicrosomal antibody

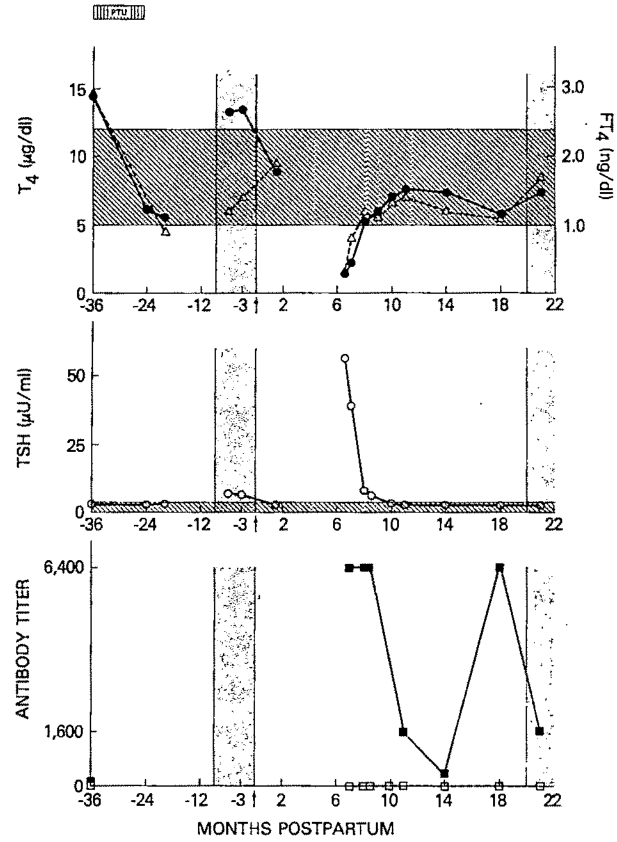

Fig. 6. Thyroid function tests and antibody levels in Case 5. Legend as in Fig. 1. Propylthiouracil *(PTU)* was administered during the period indicated in the upper panel. Pregnancy is indicated by the stippled areas; delivery is indicated by the arrows.

titers (Fig. 5). Replacement thyroid hormone was given for 2½ months, with clinical improvement. Triiodothyronine was then withheld, and she remained euthyroid until March, 1978, when she developed headaches and tachycardia, without tremor, diarrhea, anxiety, or neck pain. Her thyroid hormone levels were elevated (T_4, 18.1 μg/dl, T_3, 240 ng/dl), but the radioiodine uptake was 1%. Within 3 weeks, her thyroid hormone levels were normal; and after another 3 weeks, she was sluggish, fatigued, and chemically hypothyroid (T_4, 0.6 μg/dl; FT_4, 0.2 ng/dl; T_3, 70 ng/dl; TSH, greater than 100 μU/ml). Radioiodine uptake was then 11%, and scanning revealed an irregular thyroid without focal defects. During this period of hypothyroidism, her antibody titer rose substantially. A fine-needle aspiration biopsy at that time revealed many lymphocytes and histiocytes; a diagnosis of lymphocytic thyroiditis was made. After 1 week of prednisone, the goiter decreased in size by 40%, coincident with a fall in TSH to 32 μU/ml and a rise in thyroid hormones (T_4, 3.4 μg/dl; FT_4, 0.9 ng/dl; T_3, 141 ng/dl). L-Thyroxine replacement was begun after she continued to be hypothyroid for 6 weeks. Four months after starting thyroxine replacement, she became pregnant and her antibody titers fell. She is currently being followed on thyroxine replacement through an otherwise uncomplicated pregnancy. Both of her children have grown and developed normally, without thyroid dysfunction. There is no family history of endocrine or autoimmune disease.

Case 5. A 34-year-old white woman presented in October, 1974, with typical signs and symptoms of thyrotoxicosis. Her thyroid gland was diffusely enlarged to twice normal size, and she had periorbital edema without exophthalmos, ophthalmoplegia, or pretibial myxedema. Radioiodine uptake was 72% at 24 hours. T_4 was 14.4 μg/dl (normal, 4.6 to 10.7); FT_4 was 2.9 ngdl; T_3 was 389 ng/dl (normal, 60 to 190); and TSH was 3.1 μU/ml (normal, less than 10) (Fig. 6). She was treated with propylthiouracil, and good control was rapidly established. Use of the drug was discontinued after 10 months. She then remained euthyroid despite the presence of a goiter, diffusely enlarged to 1½ times normal. Her periorbital edema gradually cleared during this time. She became pregnant approximately 6 months after discontinuing the propylthiouracil and was euthyroid clinically and chemically during the pregnancy. She continued to be well until 6 months after delivery, when she complained of a gain in weight, puffiness of the face, fatigue, and lethargy. Her thyroid gland had increased to twice normal size. She was hypothyroid; T_4, 1.4 μg/dl; FT_4, 0.3 ng/dl; T_3, 88 ng/dl; and TSH, 56 μU/ml. Her antimicrosomal antibodies became positive. She refused aspiration biopsy but agreed to be followed without replacement therapy, and within 3 to 4 months, her basal thyroid function tests were normal, with no goiter. She was euthyroid for 8 months before becoming pregnant again, and has been euthyroid without goiter through the first trimester. There is no family history of endocrine or autoimmune disease. The infant has grown and developed normally, without thyroid dysfunction.

Comment

Postpartum thyroiditis has been recognized as a distinct entity in recent years.[3-5] The first description was from Japan, reported by Amino and colleagues,[3] who described 25 episodes of postpartum hypothyroidism in 23 patients, only three of whom were permanently hypothyroid. All patients studied had elevated antithyroid antibodies, and the single patient in whom biopsy was performed had lymphocytic thyroiditis. Fourteen patients had no history of thyroid disease before pregnancy, and although the authors ascribed the syndrome to autoimmune thyroiditis, they stressed the transient hypothyroidism. Ginsberg and Walfish[5] reported on five patients in Canada who had transient thyrotoxicosis and painless goiter that occurred 1 to 6 months after delivery. All but one patient had low ^{131}I uptake while hyperthyroid, and three women subsequently became hypothyroid, one permanently. In two patients the aspiration biopsy findings were interpreted as "consistent with chronic thyroiditis,"[5] and a third patient had elevated antithyroid antibodies. The authors concluded that their patients probably had subacute thyroiditis. Hoffbrand and Webb[4] reported on three patients in England who had postpartum goiter and hypothyroidism. At the time of thyroid dysfunction, all of them had elevated antithyroid antibodies; these resolved within 14 months of delivery, as did the goiter and the hypothyroidism.

Our American patients demonstrate that, although the postpartum hypothyroidism may be transient, the thyroid dysfunction often occurs in the context of chronic thyroid disease. Although it is unclear whether thyroiditis was present before gestation or occurred de novo post partum, all of our patients have evidence of persistent thyroid disease. Patient 5 had apparent Graves' hyperthyroidism and ophthalmopathy, with an elevated radioiodine uptake, 2½ years before postpartum hypothyroidism. Although Patient 1 developed a goiter during pregnancy, the other patients had no evident thyroid disease before delivery. Nevertheless, all our patients, except Patient 5, have evidence of chronically altered thyroid function, with residual goiter, compensated hypothyroidism, and/or frank hypothyroidism. Two of our patients have family histories of autoimmune disease, thus suggesting a genetic factor. Nevertheless, none of our patients has evidence of the HLA allele A-1/B-8 (data not shown). This has been associated with a variety of autoimmune disorders, including Graves' disease and idiopathic hypothyroidism, but has generally not been associated with Hashimoto's disease.[8] Thus, there is some evidence that our patients are genetically most like others with thyroiditis. In this regard, Patient 3 is especially interesting, since not only does she have a positive family history and possible systemic lupus erythematosus, but she also lacked all manifestations of thyroid dysfunction in the only postpartum period during which she was treated with prednisone.

Although the long-term course is presently unknown, Patients 3 and 4 may be at risk for recurrence of a similar syndrome either with or without intervening pregnancy. These patients demonstrate the futility, in many cases, of attempts to rigidly classify the types of thyroiditis. In the past, our patients might have been labeled as having subacute,[5] chronic,[2] "painless"[9] lymphocytic, or "silent" thyroiditis. Yet their disease appears to be a portion of the broad spectrum of autoimmune thyroid dysfunction.

Fine-needle aspiration cytology revealed lymphocytes and histiocytes in all four of our patients in whom biopsies were performed. Three patients also had germinal center cells (Fig. 2). No biopsy specimen showed multinucleated giant cells or many polymorphonuclear leukocytes. These cytologic findings are consistent with those in patients who had surgically proved lymphocytic thyroiditis.[10] In the context of our patients' high titer of antithyroid antibodies that correlated with the degree of thyroid dysfunction, this pathologic evidence suggests that postpartum thyroiditis is an autoimmune disorder. However, we recognize that these cytologic findings could be associated with a variety of pathogenetic mechanisms.

We treated two of our patients with a 1-week course of prednisone before instituting suppressive therapy with thyroxine.[7] The rapid reduction in the size of their goiters was another indication of the autoimmune nature of their thyroid disease. Nevertheless, we used prednisone to reduce the size of the goiter only before thyroxine therapy and for only 1 week. We have not seen any serious side effects with such brief therapy. In Patient 3, prednisone was given for many months by other physicians solely for the therapy of presumed systemic lupus erythematosus. We believe that the prolonged use of potentially hazardous glucocorticoids is contraindicated in the treatment of chronic thyroiditis.

Why does thyroiditis apparently "flare" after delivery? Pregnancy is a state of relative immune incompetence which allows survival of the antigenically dissimilar fetus. Gestation is associated with a decrease in the number and function of circulating T cells,[11] as well as depressed neutrophil chemotaxis and phagocytosis.[12] These effects may be mediated by humoral factors.[13] Humoral immunity is likewise altered during pregnancy, with lowered levels of IgA and IgG.[14]

Perhaps because of these alterations in immune function, autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, and idiopathic thrombocytopenic purpura,[15] may improve early in pregnancy and frequently worsen after delivery. Women with preexisting Graves' disease or autoimmune thyroiditis often have a dramatic fall in antithyroid antibody titers during the second half of gestation, which then return to the previously high levels after delivery[15]; more than half of these patients have a transient postpartum "rebound" to titers higher than those before pregnancy. This characteristically occurs 3 to 4 months after delivery and can occur after abortion, as well.[3, 15]

The patients reported on here demonstrate this autoimmune "flare" phenomenon, with a temporal relationship of the thyroiditis to pregnancy, thus suggesting that subtle alterations in immune function may be pathogenic in lymphocytic thyroiditis. In this context, the roles of environmental factors in inciting or promoting autoimmune thyroiditis are unclear.

We are likewise uncertain why postpartum thyroiditis has been recognized only recently, although the symptoms and signs of it are subtle and may be fleeting, so that the syndrome may have been overlooked. However, it is more likely that the prevalence of thyroiditis is increasing, especially that which is accompanied by hyperthyroidism. Lymphocytic thyroiditis with spontaneously resolving hyperthyroidism dramatically increased between 1970 and 1976 at one clinic, and now accounts for almost one quarter of all hyperthyroidism seen there.[16]

The incidence of postpartum thyroiditis is unknown, since the clinical manifestations of it occur at a time when few women are under active medical surveillance, and the symptoms of hypothyroidism could be easily dismissed, especially in the context of childbearing. Likewise, the apparent transience of symptoms may result in affected women not seeking care. Therefore, the clinician must pay careful attention to women with goiters that appear during pregnancy or after delivery, and anyone with a history of thyroid dysfunction before pregnancy must be closely followed. We suspect that this entity is commonly overlooked by patients and physicians, and may be a significant cause of morbidity in the reproductive years.

We wish to thank Dr. Elizabeth Chu for her kind assistance in the pathologic diagnosis of aspiration biopsy specimens, and for Fig. 2.

Addendum

Patient 3 recently gave birth to her fourth child. She was euthyroid, with moderately elevated TSH levels, throughout the pregnancy. At 6 weeks post partum, there was no change in the small goiter, but the serum thyroid hormones were elevated. She thus appears to have another recurrence of postpartum thyroiditis. We have also encountered two additional cases of biopsy-proved postpartum lymphocytic thyroiditis. Both women were euthyroid through pregnancy, developed high serum thyroid hormone levels at 6 and 12 weeks post partum, respectively, and have become euthyroid within 6 more weeks.

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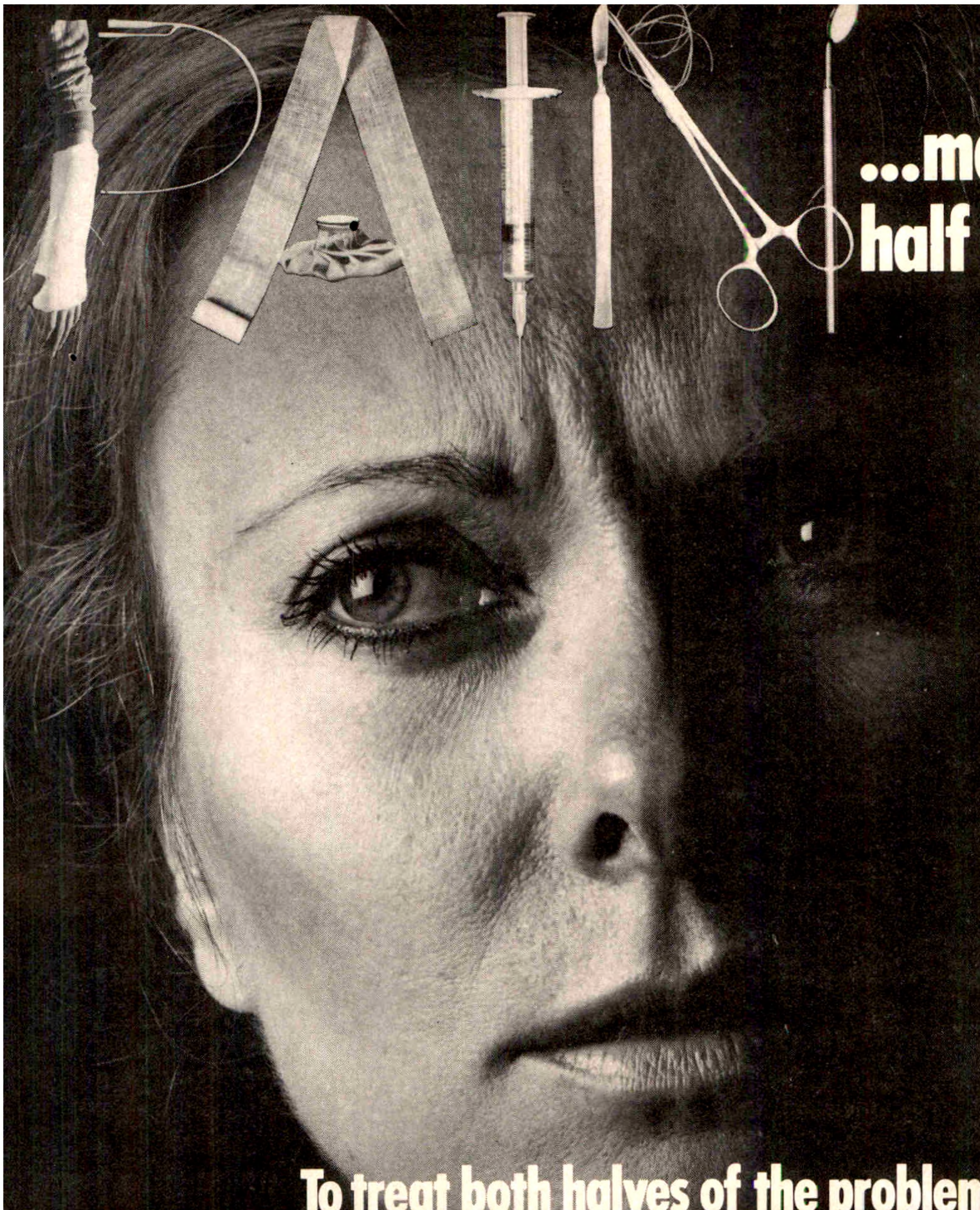

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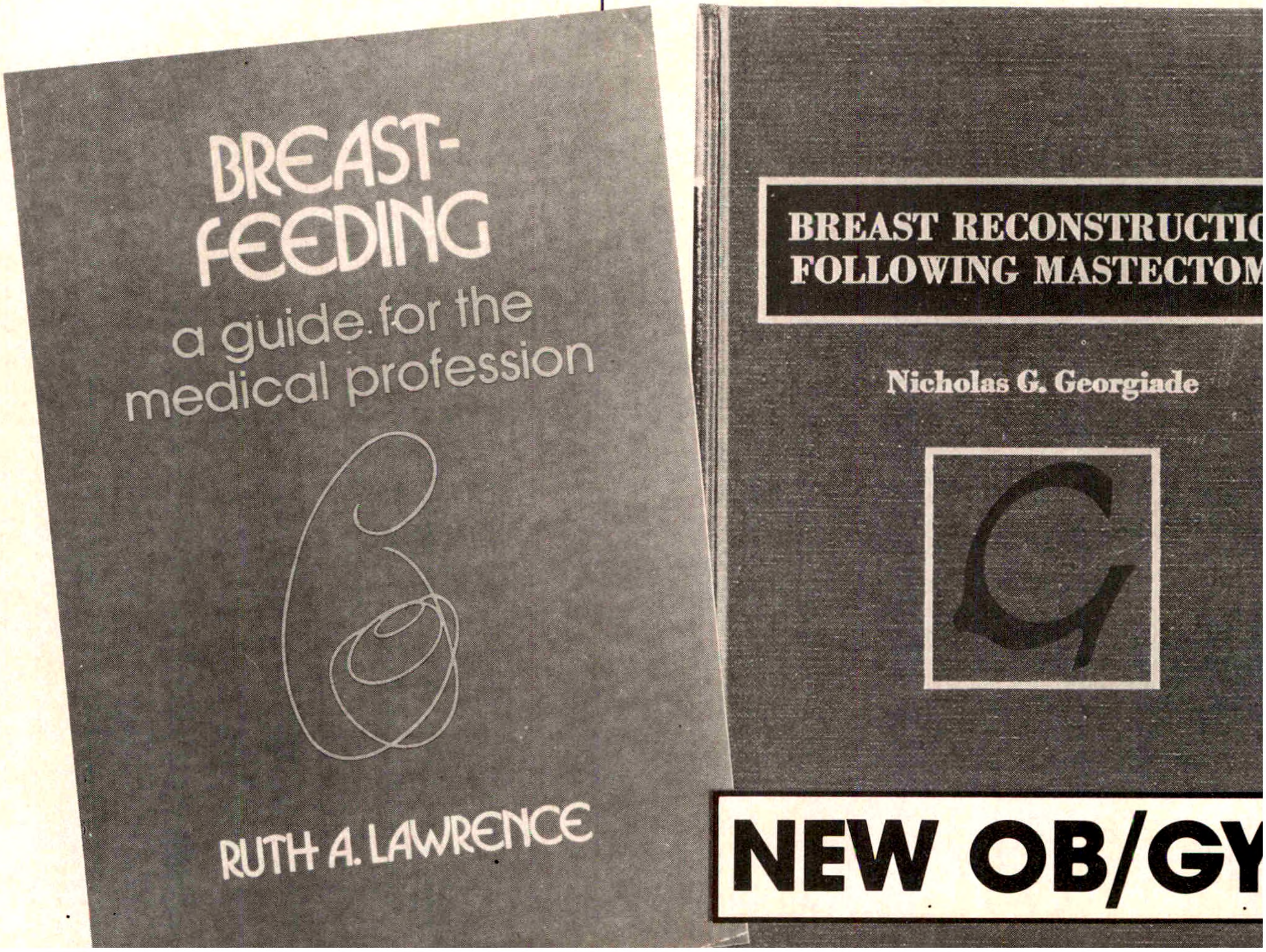

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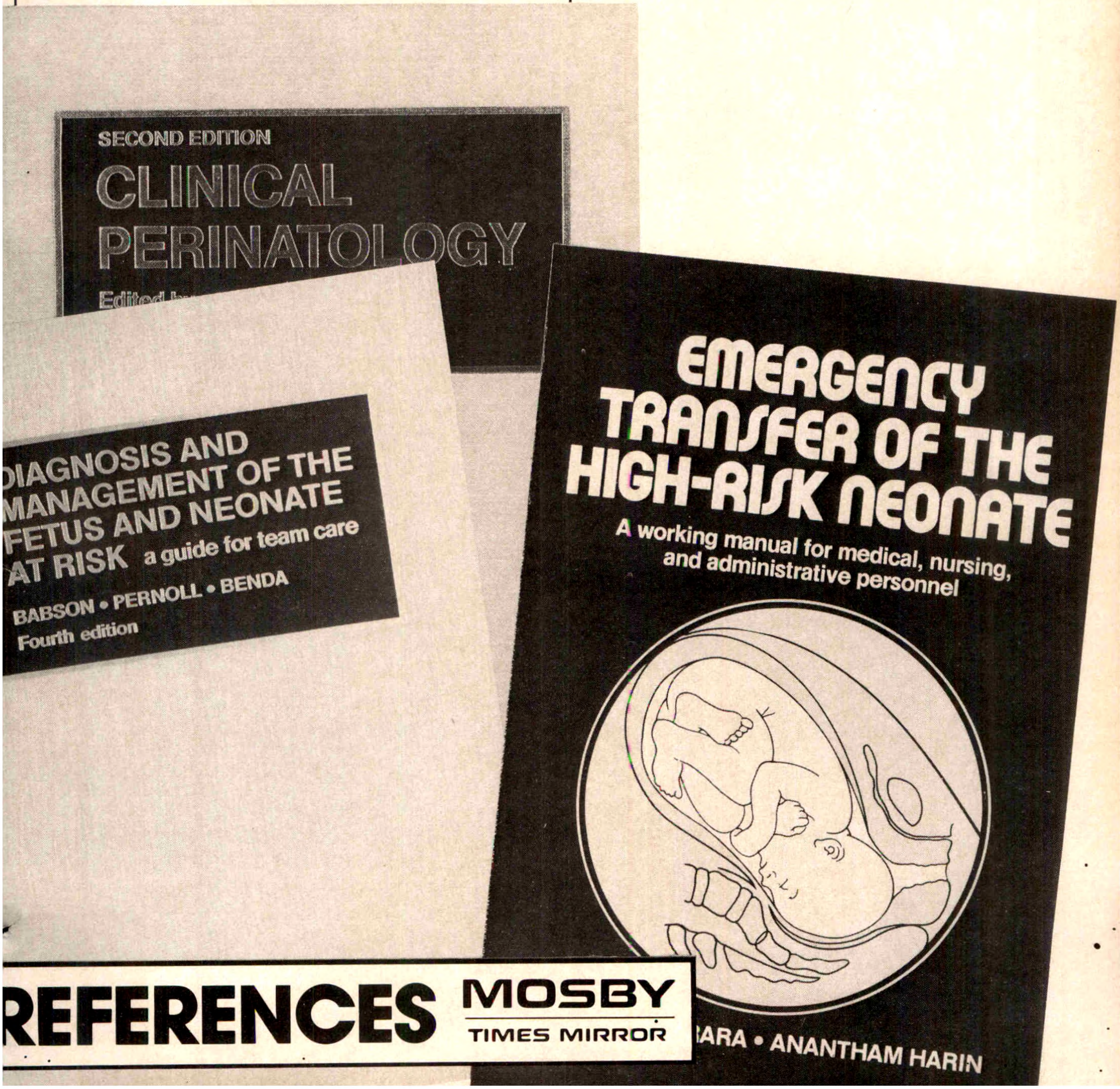
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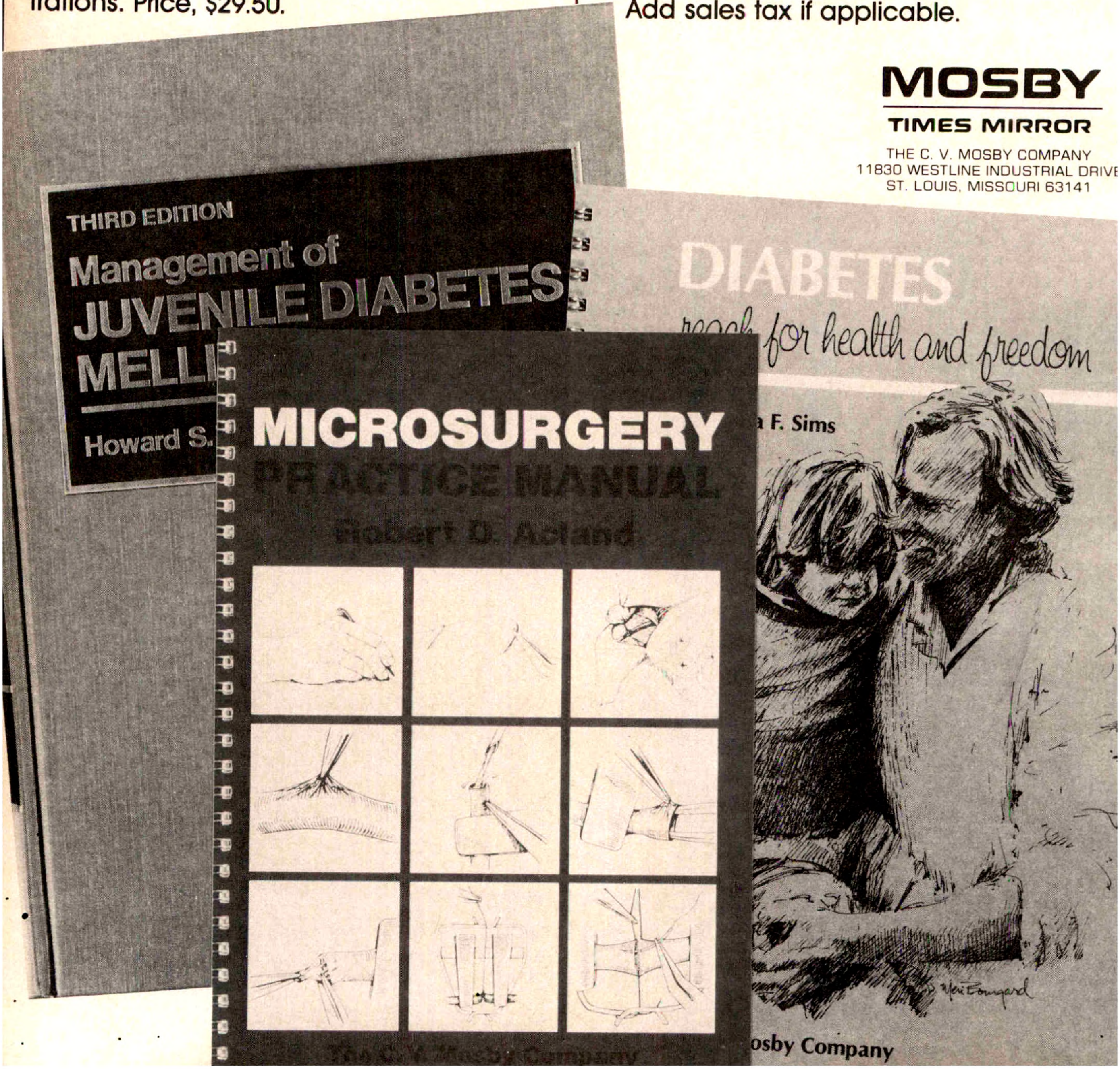

sone, 10 mg four times a day for 1 week, was given to shrink the thyroid.[7] This resulted in a 30% decrease in the size of the goiter. Administration of L-thyroxine, 0.2 mg daily, was then begun. Symptoms resolved within 3 weeks, and by 10 months post partum, the thyroid was barely palpable. Aspiration biopsy at that time revealed many lymphocytes, histiocytes, and occasional germinal center cells; a diagnosis of lymphocytic thyroiditis was made. Therapy was then changed to L-triiodothyronine, 25 μg three times a day, which was discontinued 11 months post partum. The patient continued to feel well, and within 1 more month the thyroid function tests were normal. The TSH response to thyrotropin-releasing hormone (TRH) was also normal, rising from a basal 3.9 μU/ml to 25.4 μU/ml at 30 minutes. The thyroid was moderately firm and slightly larger than normal. The patient has remained euthyroid for 6 months, but the small goiter persists. Family history includes rheumatoid arthritis in the patient's mother, but no endocrine disease. The infant has grown and developed normally, without thyroid dysfunction.

Case 3. A 29-year-old white woman gave birth in September, 1972, after her first pregnancy. She had no known signs or symptoms of thyroid disease until 3 months post partum, when she became fatigued and noticed a visible, painless goiter. One month later, thyroid function tests showed: T_4, 1.5 μg/dl (normal 4.5 to 10.7) and T_3RU, 24%. Twenty-four-hour ^{131}I uptake was 21%, with a homogeneous, moderately enlarged thyroid on scan. Treatment was begun with L-thyroxine, 0.1 mg daily. The patient discontinued this 2 months later, without any further fatigue, and the goiter disappeared within 6 months without therapy. In October, 1974, she had visual and auditory hallucinations and arthralgias, but no rash or fever. Testing for antinuclear antibody was positive. The diagnosis of systemic lupus erythematosus was made, and prednisone, 20 mg daily, was begun, with prompt resolution of symptoms. She gave birth again in February, 1976, remaining on prednisone throughout her pregnancy and postpartum period in doses of 20 to 60 mg daily. She had no clinical thyroid dysfunction, and no thyroid function tests were performed during or after this pregnancy. In September, 1976, she was referred to the NIH, where an extensive serologic examination was negative for systemic lupus. The prednisone was tapered and discontinued in June, 1977, without complications.

The patient gave birth again in May, 1978. Six weeks post partum, she noticed trembling, headache, insomnia, and increased appetite. She had no neck pain. Thyroid hormones were elevated (T_4, 15μg/dl; T_3RU, 37%), and 24-hour ^{131}I uptake was 6%. The symptoms cleared within 3 weeks, only to be followed 2 months later by clinical and chemical hypothyroidism: T_4, 4.3 μg/dl; TSH, 40 μU/ml (Fig. 4). Elevated antimicrosomal antibodies were found, and L-thyroxine, 50 μg daily, was begun. Aspiration biopsy in January, 1979, revealed lymphocytes, histiocytes, plasma cells, and germinal center cells; a diagnosis of lymphocytic thyroiditis was made. The thyroxine was then withheld, and the patient remained asymptomatic. Since then, she has had a barely palpable, moderately firm thyroid, without nodularity. Thyroid hormones have remained normal through 14 months post partum (T_4, 5.2 μg/dl; FT_4, 1.3 ng/dl; T_3, 165 ng/dl). TSH is 7.9 μU/ml, and hyperresponds to TRH (37.2 at 30 minutes). Family history reveals idiopathic hypothyroidism in her mother and a brother, but no other endocrine or autoimmune disease. All children have been euthyroid, and have grown and developed normally.

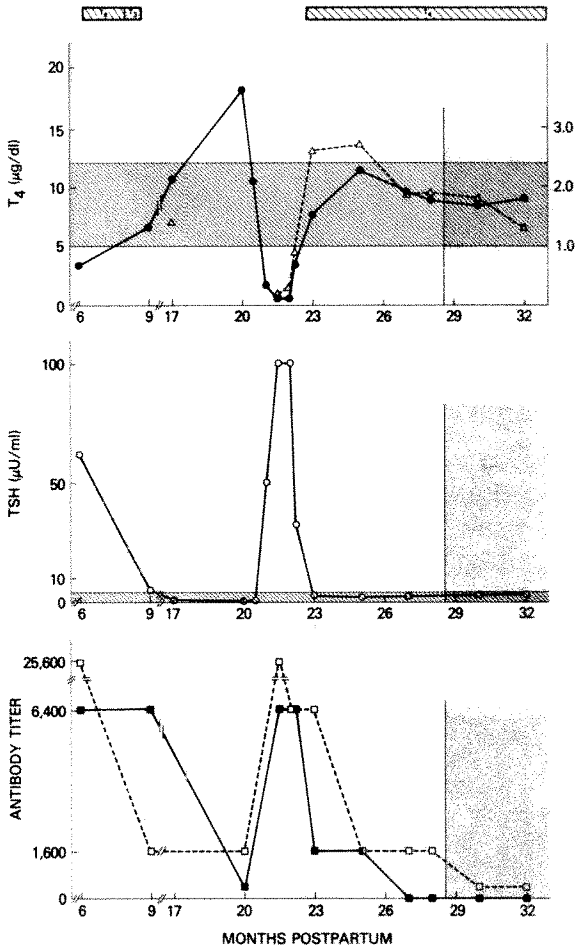

Fig. 5. Thyroid function tests and antibody levels after the second delivery in Case 4. Legend as in Fig. 1. The patient is currently pregnant, as indicated by the stippled areas.

Case 4. A 26-year-old white woman gave birth to her second child in July, 1976. She was well until she noticed a goiter, increased weight, and fatigue 6 months later. There was no previous history of thyroid disease. She was hypothyroid (T_4, 3.4 μg/dl; T_3, 75 ng/dl [normal, 80 to 160]; TSH, 62 μU/ml), with elevated antithyroglobulin and antimicrosomal antibody

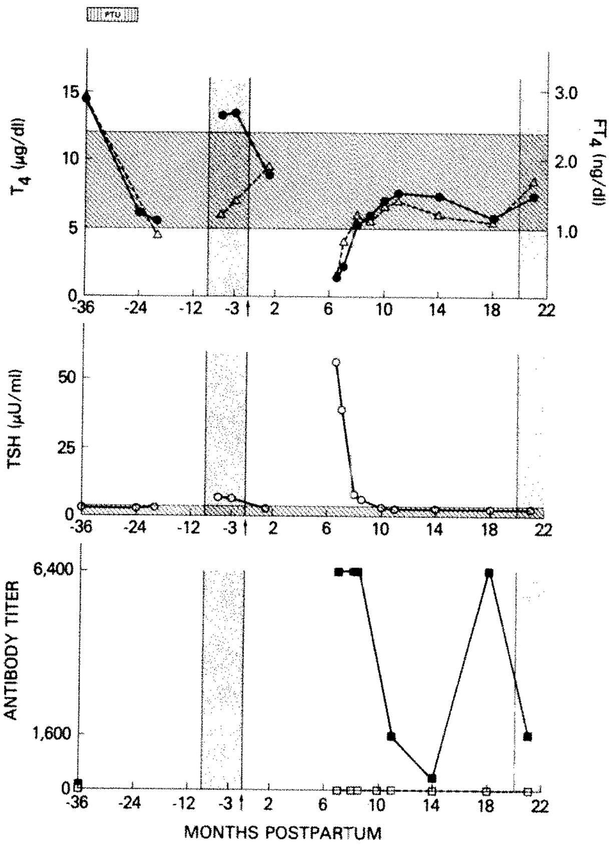

Fig. 6. Thyroid function tests and antibody levels in Case 5. Legend as in Fig. 1. Propylthiouracil *(PTU)* was administered during the period indicated in the upper panel. Pregnancy is indicated by the stippled areas; delivery is indicated by the arrows.

titers (Fig. 5). Replacement thyroid hormone was given for 2½ months, with clinical improvement. Triiodothyronine was then withheld, and she remained euthyroid until March, 1978, when she developed headaches and tachycardia, without tremor, diarrhea, anxiety, or neck pain. Her thyroid hormone levels were elevated (T$_4$, 18.1 μg/dl, T$_3$, 240 ng/dl), but the radioiodine uptake was 1%. Within 3 weeks, her thyroid hormone levels were normal; and after another 3 weeks, she was sluggish, fatigued, and chemically hypothyroid (T$_4$, 0.6 μg/dl; FT$_4$, 0.2 ng/dl; T$_3$, 70 ng/dl; TSH, greater than 100 μU/ml). Radioiodine uptake was then 11%, and scanning revealed an irregular thyroid without focal defects. During this period of hypothyroidism, her antibody titer rose substantially. A fine-needle aspiration biopsy at that time revealed many lymphocytes and histiocytes; a diagnosis of lymphocytic thyroiditis was made. After 1 week of prednisone, the goiter decreased in size by 40%, coincident with a fall in TSH to 32 μU/ml and a rise in thyroid hormones (T$_4$, 3.4 μg/dl; FT$_4$, 0.9 ng/dl; T$_3$, 141 ng/dl). L-Thyroxine replacement was begun after she continued to be hypothyroid for 6 weeks. Four months after starting thyroxine replacement, she became pregnant and her antibody titers fell. She is currently being followed on thyroxine replacement through an otherwise uncomplicated pregnancy. Both of her children have grown and developed normally, without thyroid dysfunction. There is no family history of endocrine or autoimmune disease.

Case 5. A 34-year-old white woman presented in October, 1974, with typical signs and symptoms of thyrotoxicosis. Her thyroid gland was diffusely enlarged to twice normal size, and she had periorbital edema without exophthalmos, ophthalmoplegia, or pretibial myxedema. Radioiodine uptake was 72% at 24 hours. T$_4$ was 14.4 μg/dl (normal, 4.6 to 10.7); FT$_4$ was 2.9 ngdl; T$_3$ was 389 ng/dl (normal, 60 to 190); and TSH was 3.1 μU/ml (normal, less than 10) (Fig. 6). She was treated with propylthiouracil, and good control was rapidly established. Use of the drug was discontinued after 10 months. She then remained euthyroid despite the presence of a goiter, diffusely enlarged to 1½ times normal. Her periorbital edema gradually cleared during this time. She became pregnant approximately 6 months after discontinuing the propylthiouracil and was euthyroid clinically and chemically during the pregnancy. She continued to be well until 6 months after delivery, when she complained of a gain in weight, puffiness of the face, fatigue, and lethargy. Her thyroid gland had increased to twice normal size. She was hypothyroid; T$_4$, 1.4 μg/dl; FT$_4$, 0.3 ng/dl; T$_3$, 88 ng/dl; and TSH, 56 μU/ml. Her antimicrosomal antibodies became positive. She refused aspiration biopsy but agreed to be followed without replacement therapy, and within 3 to 4 months, her basal thyroid function tests were normal, with no goiter. She was euthyroid for 8 months before becoming pregnant again, and has been euthyroid without goiter through the first trimester. There is no family history of endocrine or autoimmune disease. The infant has grown and developed normally, without thyroid dysfunction.

Comment

Postpartum thyroiditis has been recognized as a distinct entity in recent years.[3-5] The first description was from Japan, reported by Amino and colleagues,[3] who described 25 episodes of postpartum hypothyroidism in 23 patients, only three of whom were permanently hypothyroid. All patients studied had elevated antithyroid antibodies, and the single patient in whom biopsy was performed had lymphocytic thyroiditis. Fourteen patients had no history of thyroid disease before pregnancy, and although the authors ascribed the syndrome to autoimmune thyroiditis, they stressed the transient hypothyroidism. Ginsberg and Walfish[5] reported on five patients in Canada who had transient thyrotoxicosis and painless goiter that occurred 1 to 6 months after delivery. All but one patient had low ^{131}I uptake while hyperthyroid, and three women subsequently became hypothyroid, one permanently. In two patients the aspiration biopsy findings were interpreted as "consistent with chronic thyroiditis,"[5] and a third patient had elevated antithyroid antibodies. The authors concluded that their patients probably had subacute thyroiditis. Hoffbrand and Webb[4] reported on three patients in England who had postpartum goiter and hypothyroidism. At the time of thyroid dysfunction, all of them had elevated antithyroid antibodies; these resolved within 14 months of delivery, as did the goiter and the hypothyroidism.

Our American patients demonstrate that, although the postpartum hypothyroidism may be transient, the thyroid dysfunction often occurs in the context of chronic thyroid disease. Although it is unclear whether thyroiditis was present before gestation or occurred de novo post partum, all of our patients have evidence of persistent thyroid disease. Patient 5 had apparent Graves' hyperthyroidism and ophthalmopathy, with an elevated radioiodine uptake, 2½ years before postpartum hypothyroidism. Although Patient 1 developed a goiter during pregnancy, the other patients had no evident thyroid disease before delivery. Nevertheless, all our patients, except Patient 5, have evidence of chronically altered thyroid function, with residual goiter, compensated hypothyroidism, and/or frank hypothyroidism. Two of our patients have family histories of autoimmune disease, thus suggesting a genetic factor. Nevertheless, none of our patients has evidence of the HLA allele A-1/B-8 (data not shown). This has been associated with a variety of autoimmune disorders, including Graves' disease and idiopathic hypothyroidism, but has generally not been associated with Hashimoto's disease.[8] Thus, there is some evidence that our patients are genetically most like others with thyroiditis. In this regard, Patient 3 is especially interesting, since not only does she have a positive family history and possible systemic lupus erythematosus, but she also lacked all manifestations of thyroid dysfunction in the only postpartum period during which she was treated with prednisone.

Although the long-term course is presently unknown, Patients 3 and 4 may be at risk for recurrence of a similar syndrome either with or without intervening pregnancy. These patients demonstrate the futility, in many cases, of attempts to rigidly classify the types of thyroiditis. In the past, our patients might have been labeled as having subacute,[5] chronic,[2] "painless"[9] lymphocytic, or "silent" thyroiditis. Yet their disease appears to be a portion of the broad spectrum of autoimmune thyroid dysfunction.

Fine-needle aspiration cytology revealed lymphocytes and histiocytes in all four of our patients in whom biopsies were performed. Three patients also had germinal center cells (Fig. 2). No biopsy specimen showed multinucleated giant cells or many polymorphonuclear leukocytes. These cytologic findings are consistent with those in patients who had surgically proved lymphocytic thyroiditis.[10] In the context of our patients' high titer of antithyroid antibodies that correlated with the degree of thyroid dysfunction, this pathologic evidence suggests that postpartum thyroiditis is an autoimmune disorder. However, we recognize that these cytologic findings could be associated with a variety of pathogenetic mechanisms.

We treated two of our patients with a 1-week course of prednisone before instituting suppressive therapy with thyroxine.[7] The rapid reduction in the size of their goiters was another indication of the autoimmune nature of their thyroid disease. Nevertheless, we used prednisone to reduce the size of the goiter only before thyroxine therapy and for only 1 week. We have not seen any serious side effects with such brief therapy. In Patient 3, prednisone was given for many months by other physicians solely for the therapy of presumed systemic lupus erythematosus. We believe that the prolonged use of potentially hazardous glucocorticoids is contraindicated in the treatment of chronic thyroiditis.

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Perhaps because of these alterations in immune function, autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, and idiopathic thrombocytopenic purpura,[15] may improve early in pregnancy and frequently worsen after delivery. Women with preexisting Graves' disease or autoimmune thyroiditis often have a dramatic fall in antithyroid antibody titers during the second half of gestation, which then return to the previously high levels after delivery[15]; more than half of these patients have a transient postpartum "rebound" to titers higher than those before pregnancy. This characteristically occurs 3 to 4 months after delivery and can occur after abortion, as well.[3, 15]

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We wish to thank Dr. Elizabeth Chu for her kind assistance in the pathologic diagnosis of aspiration biopsy specimens, and for Fig. 2.

Addendum

Patient 3 recently gave birth to her fourth child. She was euthyroid, with moderately elevated TSH levels, throughout the pregnancy. At 6 weeks post partum, there was no change in the small goiter, but the serum thyroid hormones were elevated. She thus appears to have another recurrence of postpartum thyroiditis. We have also encountered two additional cases of biopsy-proved postpartum lymphocytic thyroiditis. Both women were euthyroid through pregnancy, developed high serum thyroid hormone levels at 6 and 12 weeks post partum, respectively, and have become euthyroid within 6 more weeks.

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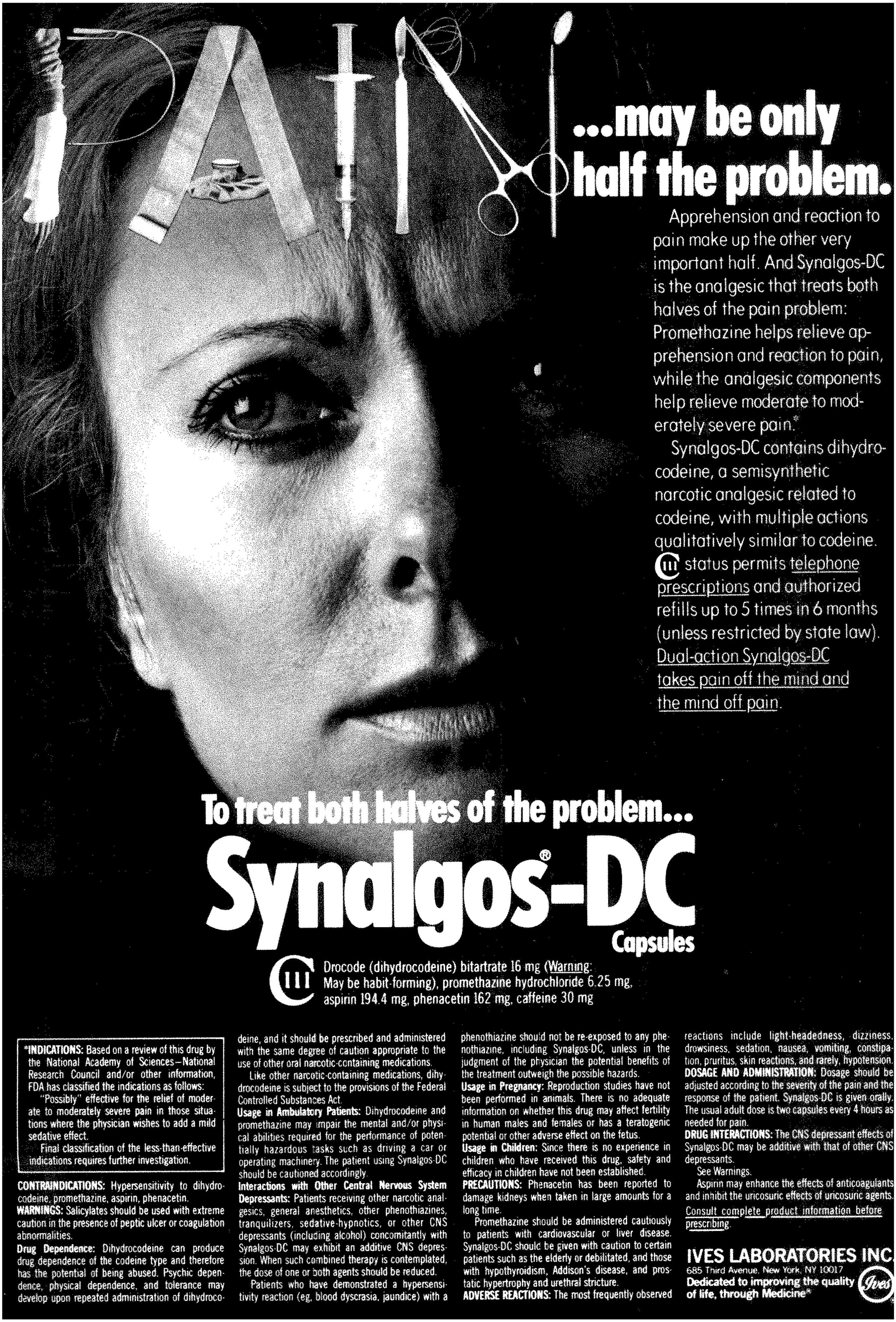

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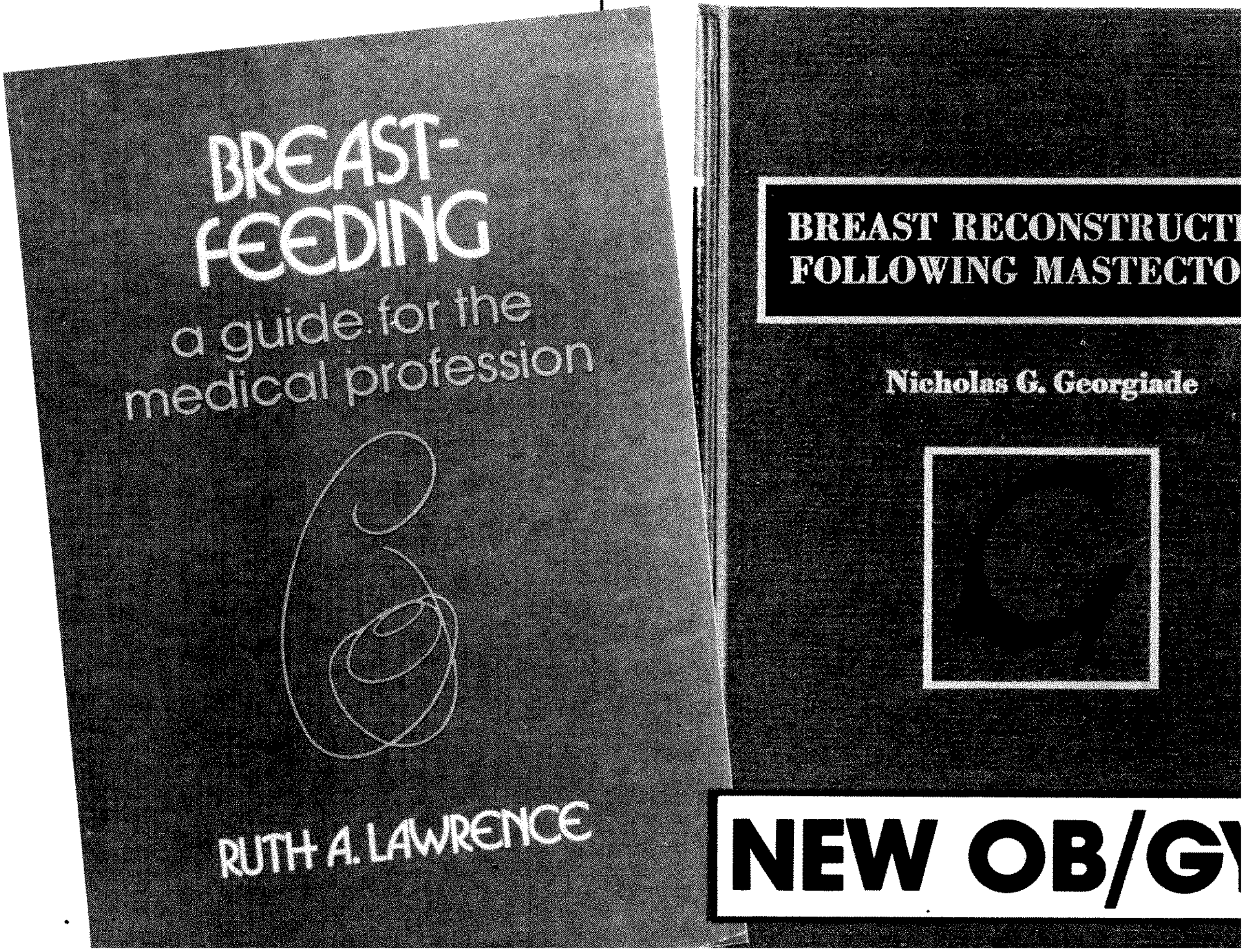

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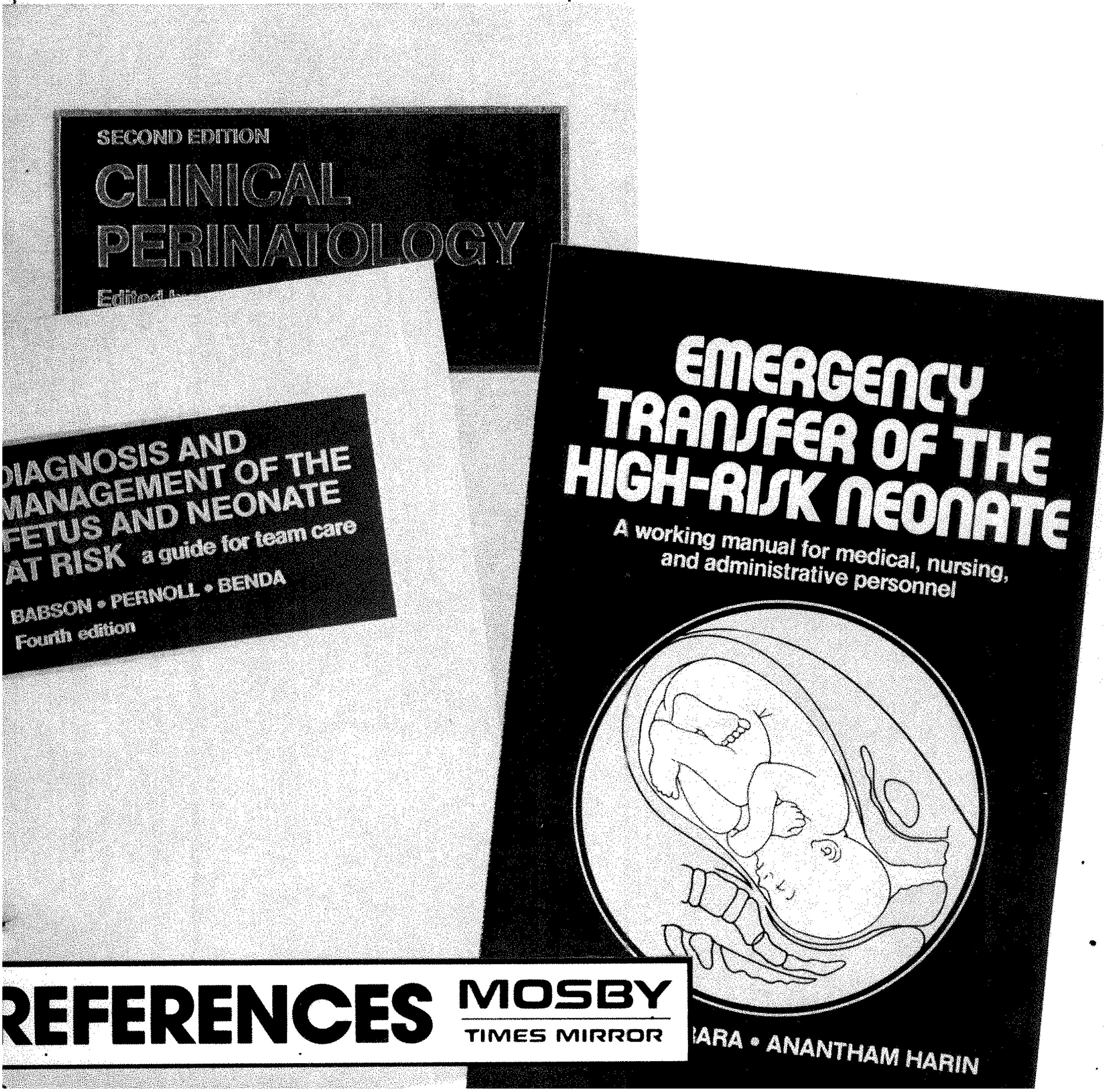

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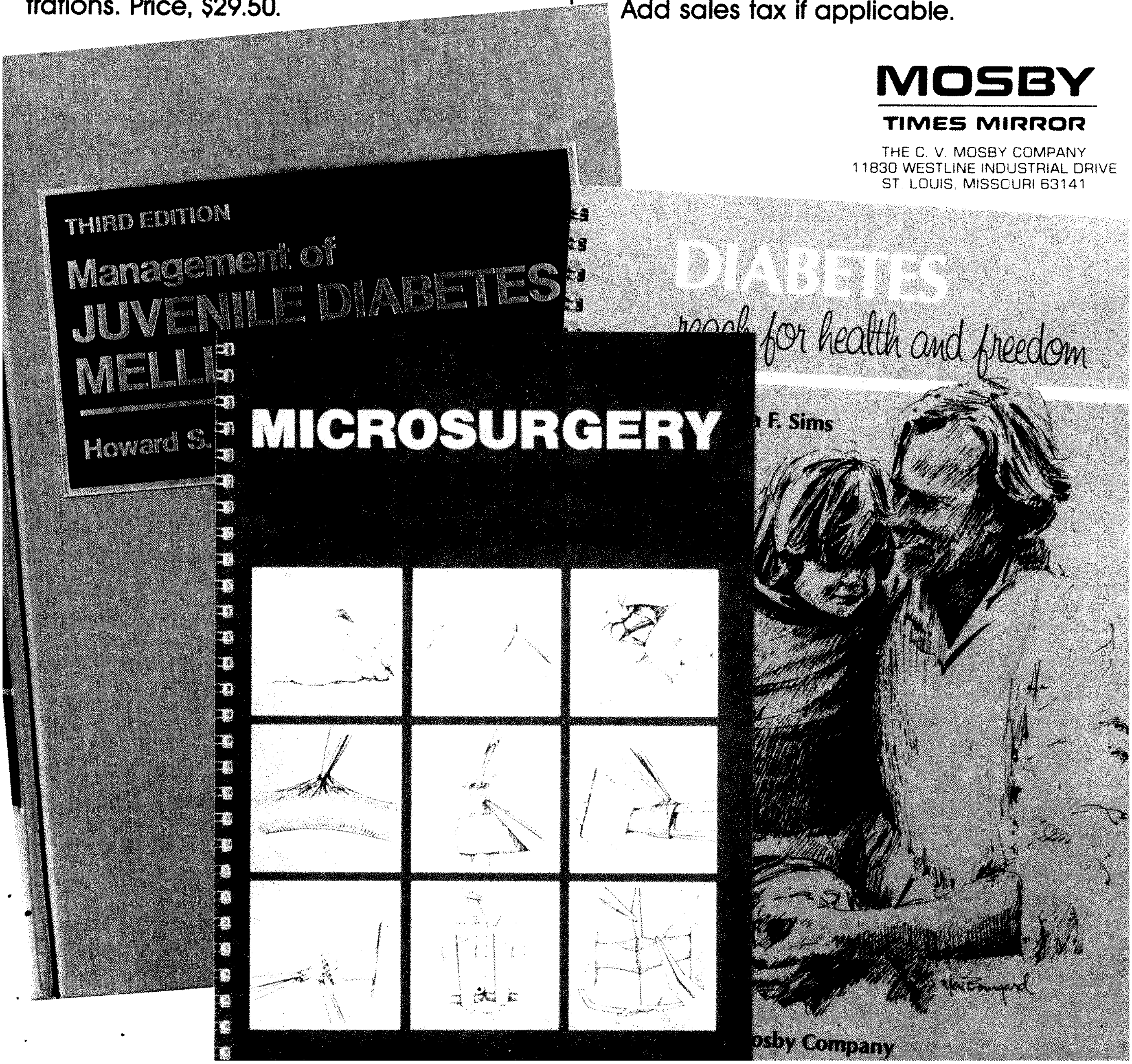

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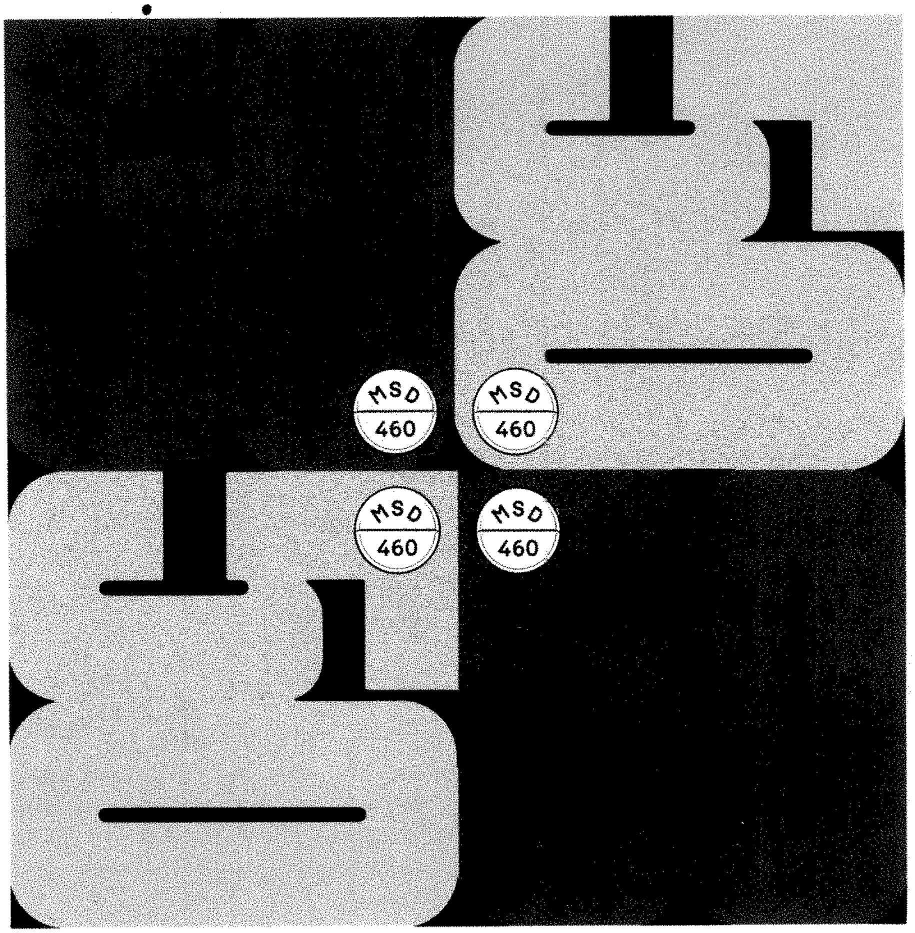

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Fetal cranial and craniocervical masses: Ultrasound characteristics and differential diagnosis

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Ultrasound is assuming an essential role in the detection of fetal cranial and spinal anomalies. Illustrated in this article is the sonographic appearance of cranial abnormalities wherein the diagnosis of encephalocele is clear cut because the anatomic defect of the neural tube is visualized. Additionally presented is a variety of cranial and craniocervical cystic masses, including meningocele, wherein the anatomic defect of the neural tube is not apparent and the diagnosis is reached by careful attention to the specific ultrasonic characteristics of each mass. (AM. J. OBSTET. GYNECOL. 138:511, 1980.)

DIAGNOSTIC ULTRASOUND is assuming a prominent role in the detection of fetal structural abnormalities[1, 2]; this status can be attributed to two main factors: technological advances in sonographic equipment, including the introduction of dynamic (real-time) imaging, and the acquisition of knowledge pertaining to the echographic appearance of structural defects. However, more time is needed before the sensitivity (proportion of affected individuals with a positive test) and the specificity (proportion of unaffected individuals with a negative test) of sonar in the diagnosis of congenital anomalies is established, because the incidence of birth defects in the general population is low and ultrasound (at least in the United States) is used only upon specific indications.

This report presents the ultrasonic characteristics and the differential diagnosis of a variety of fetal cranial and craniocervical masses, in an attempt to improve the reliability of sonography in this area of investigation.

Material and methods

The data in this report were derived from 10 pregnant women. In each pregnancy, a fetal cranial or craniocervical mass was seen ultrasonically. All fetuses were examined by either dynamic or static gray-scale

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Presented at a meeting of the Chicago Gynecological Society on November 16, 1979.

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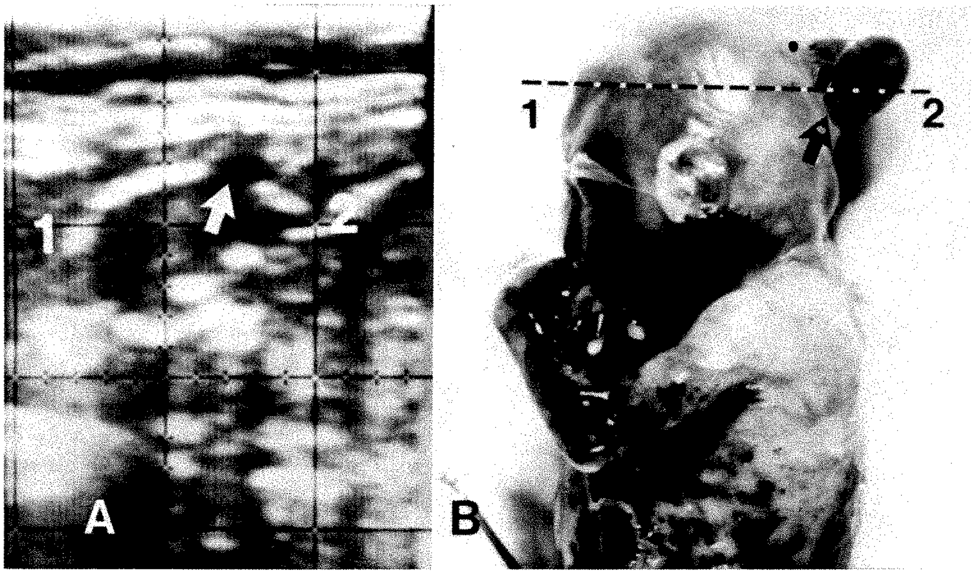

Fig. 1. *A*, *Arrow* points to a clear defect in the parietal bone echo. *B*, *Arrow* points to the defect in the fetus; the dotted line represents the plane which showed the defect on ultrasound.

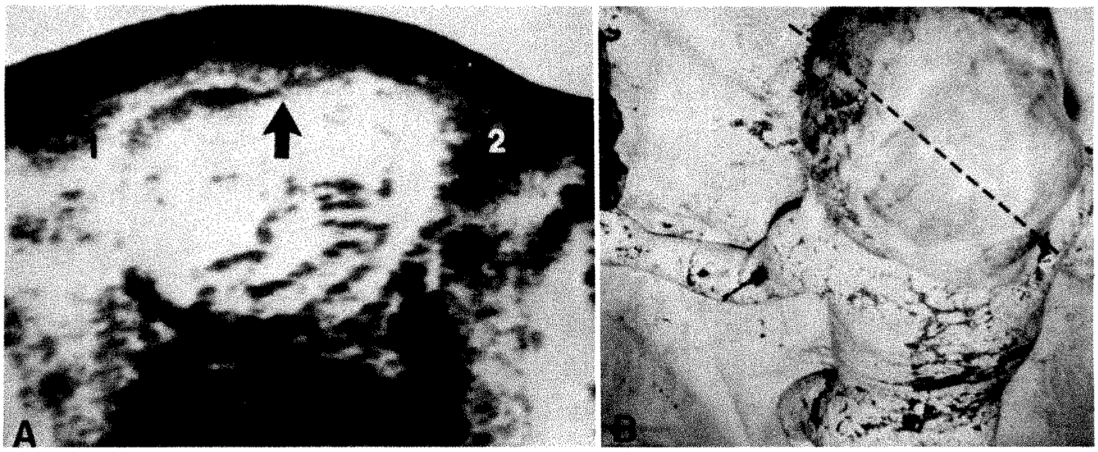

Fig. 2. *A*, *Arrow* points to the area in which the normal semicircular contour of the parietal bone echo is lost. The echo from the fetal head on the right aspect of the arrow shows an abnormal upward curvature. *B*, The *dotted line* represents the plane which showed the defect on ultrasound.

imaging, or both. The case histories and the reasons for sonography are presented below.

The first woman was at high risk of being delivered of an infant with a neural tube defect (NTD) because her previous baby had Meckel's syndrome (encephalocele, eye abnormalities, cleft palate, polydactyly, and congenital heart disease). Additionally, the α-fetoprotein (AFP) in amniotic fluid (drawn 7 days earlier) was elevated. She was scanned on two occasions, at the eighteenth and the twentieth weeks of gestation. The second examination clearly revealed a defect in the posterior aspect of the fetal skull. Consequently, she elected to have the pregnancy terminated.

The second patient was referred to this Center at approximately 36 weeks' gestation because the biparietal diameter could not be reliably measured at a local hospital. The echogram obtained here showed a large cranial bulge and disruption of the normal echopattern of the parietal skull complex. The abnormality was also confirmed radiologically. The fetus was delivered by repeat cesarean section.

Seven patients were scanned for assessment of gestational age or fetal well-being at 16 to 20 weeks' of gestation, and in all of them cystic masses adjacent to the posterior aspects of the fetal skulls were ultrasonically visualized, but no anatomic defects of the neural tube

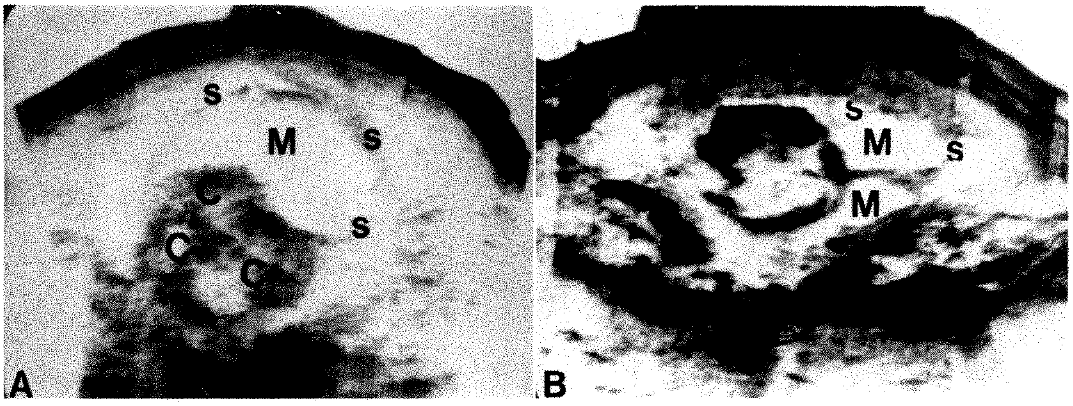

Fig. 3. *A*, A large meningocele *(M)* is seen attached to the fetal cranium *(C)*. No actual defect is observed. Notice the very thin wall *(S)* of the meningocele and the variable configuration. *B*, Notice variable configuration of meningocele *(M)* and thin wall *(S)*.

Table I. Ultrasonic characteristics and differential diagnosis of fetal cranial and craniocervical masses with no apparent defect in neural tube

		Ultrasonic characteristics of mass			
Differential diagnosis	*No. of pregnancies*	*Outer margin*	*Size and shape*	*Concomitant motion with fetal head*	*Other feasible findings*
Meningocele	2	Thin*	Variable	Yes	—
Fetal edema	2	Thick	Uniform, semicircular, simulates contour of fetal head and neck	Yes	Ascites
Intrauterine fetal death	1	Thick	Uniform, semicircular	—	Collapse of sutures
Cystic hygroma	2	Thick†	Uniform, circular or near circular; sometimes bilateral with multiple septations	Yes	Pleural or cardiac effusion, or both
Stunted twin gestational sac	1	Thin	Circular or oblong	No	—

*<3 mm.

†Thickness of margin (3 to 5 mm) may not be apparent until 18 to 20 weeks' gestation.

were apparent. All the women in this group requested to have their pregnancies terminated.

The tenth patient was a 37-year-old woman, gravida 1, with a history of infertility. She was examined ultrasonically in the first trimester of pregnancy, and twin gestational sacs and fetuses were observed. However, at the sixteenth week of gestation, prior to amniocentesis, only one fetus was seen. Additionally, a cystic mass was visualized adjacent to the fetal head, but no cranial or spinal defect was apparent. The mass was believed to be unattached to the fetus because it did not change position concomitantly with motion of the fetal head or body. Follow-up examinations of the mass showed complete resorption by the twenty-sixth week of gestation.

Results

In the first two fetuses the anatomic NTD was ultrasonically visible and the diagnosis of encephalocele made. Interestingly, the NTD was quite small in one (Fig. 1) and rather large in the other (Fig. 2). By contrast, in eight fetuses the cranial and craniocervical masses were not associated with a visible anatomic defect of the spine or the skull. Nonetheless, two of those fetuses had a meningocele (Fig. 3). The abnormalities in the other six patients (Table I) were: edema of the fetal head (in two pregnancies) (Figs. 4 and 5), fetal death (Fig. 6), unilateral and bilateral cystic hygroma, (Figs. 7 and 8), and a stunted twin gestational sac (Fig. 9).

Comment

A variety of craniocervical masses situated along the posterior or posterolateral aspects of fetuses can be visualized by ultrasound. These masses may be classified into two groups. In the first group, a small or large defect in the skull is clearly visible in conjunction with the deformity (Figs. 1 and 2) and the diagnosis of a

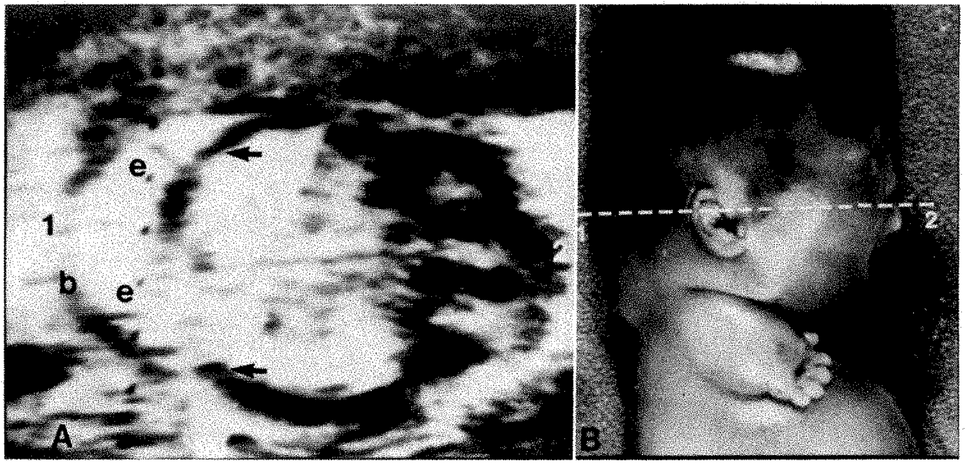

Fig. 4. *A, Arrows* point to posterior aspect of fetal skull. Marked edema *(e)* is seen posteriorly and the boundary *(b)* is quite thick. *B, Dotted line* represents the plane which showed the abnormality on ultrasound; notice marked edema of fetal neck.

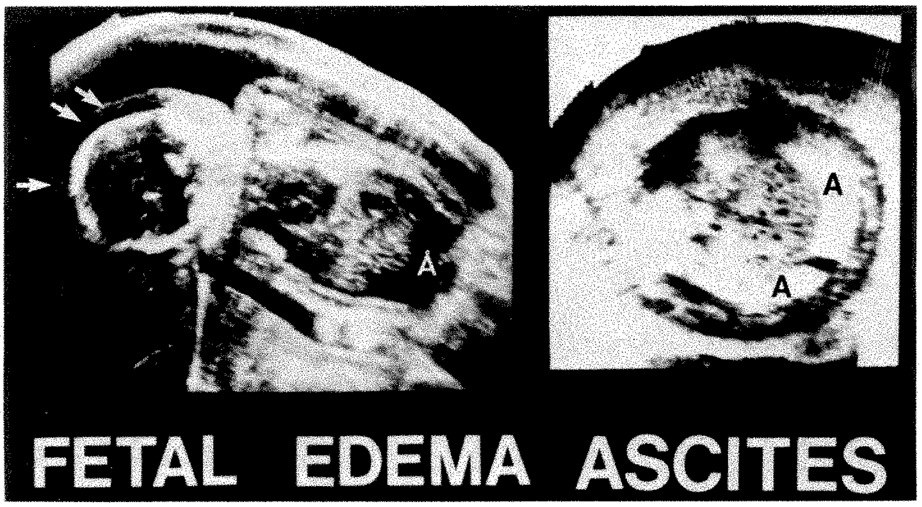

Fig. 5. *Arrows* point to halo surrounding head in a fetus with massive edema. Notice presence of ascites *(A)*, particularly in the cross-sectional view of the fetal abdomen (right side of photograph).

neural tube defect can be readily made. In the second group, the defect in the fetal vertebral column or skull is either absent or simply too small and thus not ultrasonically visible. The fetal abnormalities that produce such cystic masses are listed in Table I. The differential diagnosis is based on the following observations.

In a meningocele, the outer margin representing the thin tissues of the meninges produces a very thin echo pattern. Even when a meningocele is covered by skin, the overlying integument (possibly because of pressure from the meningocele) is quite thin (1 to 2 mm) (Fig. 3). Furthermore, a meningocele may vary in shape and size because of its fluid content and thin wall (Fig. 3). It is important to realize that the anatomic defect in the neural tube may not be ultrasonically apparent regardless of the size of the meningocele. Thus, the sonographer should not be deterred from diagnosing a meningocele in a mass with the echographic pattern of that abnormality (Table I) simply because the actual defect in the cranium or spine is not visible.

In fetal edema, the outer margin of the cystic mass (consisting of edematous skin) is quite thick and assumes a uniform semicircular shape, simulating the contour of the fetal head and neck, of which it is a part (Fig. 4). Occasionally, when edema is massive and generalized, a circular cystic halo is seen surrounding the entire fetal head (Fig. 5). In such cases, ascites is usually evident (Fig. 5).

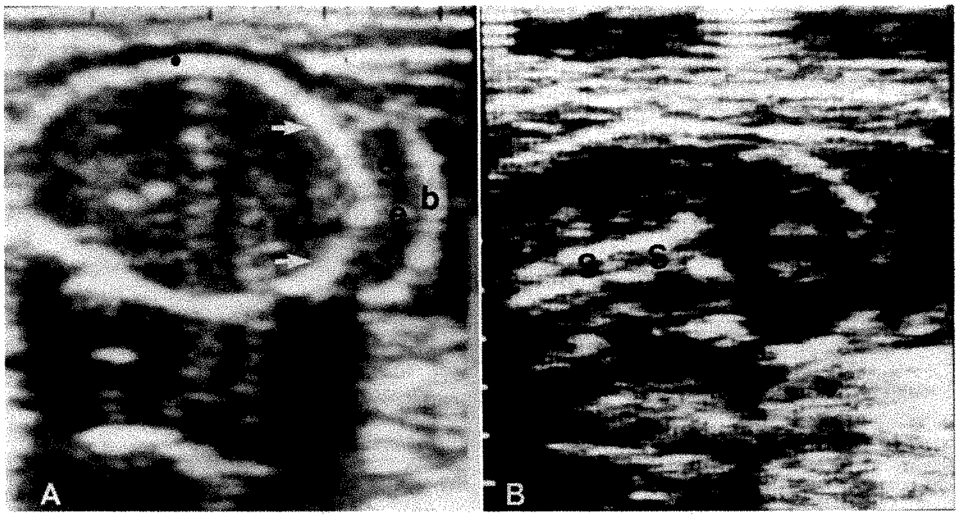

Fig. 6. *A*, *Arrows* point to posterior aspect of fetal skull in a patient with intrauterine fetal death; notice marked edema *(e)* and thick boundary *(b)*. *B*, The upper fetal spine *(s)* appears as a hollow tube and shows no defects.

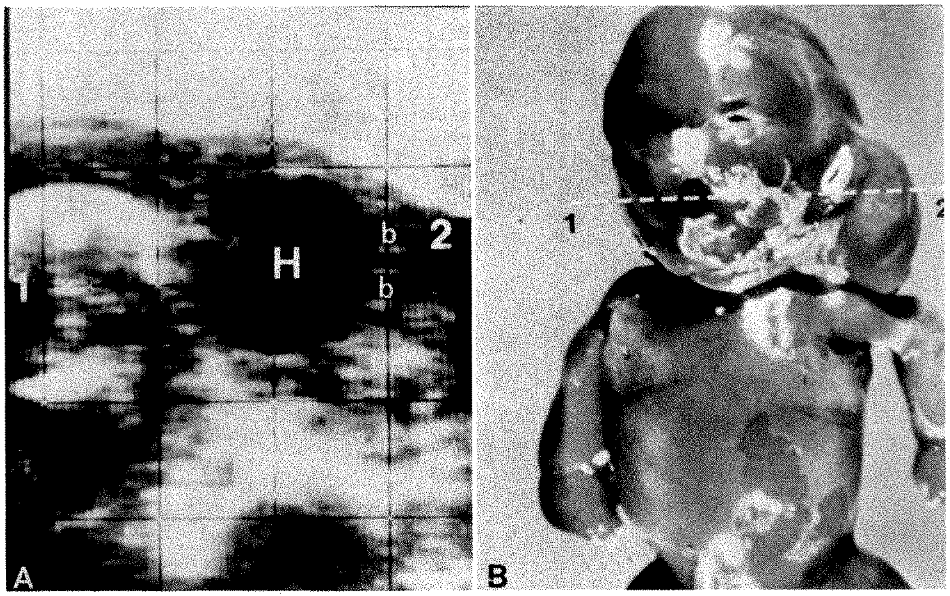

Fig. 7. *A*, A large circular mass representing a hygroma colli *(H)* is seen. Notice extension to deep aspect of fetal neck. The boundary *(b)* of the mass is quite thick, as in fetal edema and intrauterine fetal death. *B*, *Dotted line* represents the plane which showed the abnormality.

It is interesting to note that, after fetal death, echograms of the fetal head are similar to those seen with fetal edema (Fig. 6). The diagnosis of intrauterine fetal death, however, is readily made by dynamic imaging of the fetal heart.

In cystic hygroma, the outer margin of the mass appears ultrasonically thick (3 to 5 mm) by 18 to 20 weeks' gestation. However, since hygroma involves the deep lymphatics situated adjacent to the vertebral column, the mass not only protrudes externally but also extends well into the fetal neck, thereby producing a well-defined circular or nearly circular outline in the craniocervical area (Fig. 7). Furthermore, cystic hygroma is sometimes seen bilaterally, and, with proper angulation of the transducer, usually shows multiple septations representing lymphatic channels (Fig. 8).[3, 4]

It is most difficult to differentiate the echogram of a meningocele from that obtained in a twin pregnancy in

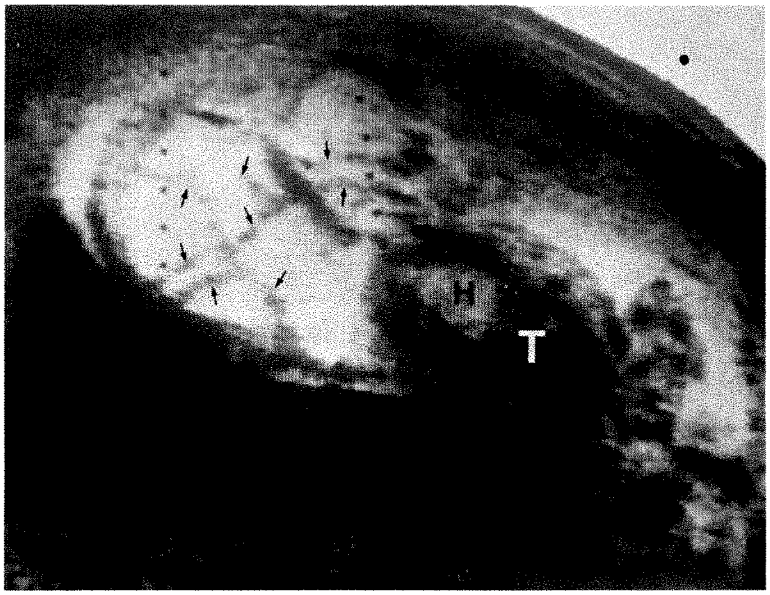

Fig. 8. Notice bilateral hygromas with thick wall and multiple septations *(arrows)*. The large masses are seen in the area of the fetal neck. Fetal trunk *(T)* and heart *(H)* are seen on right aspect of photograph.

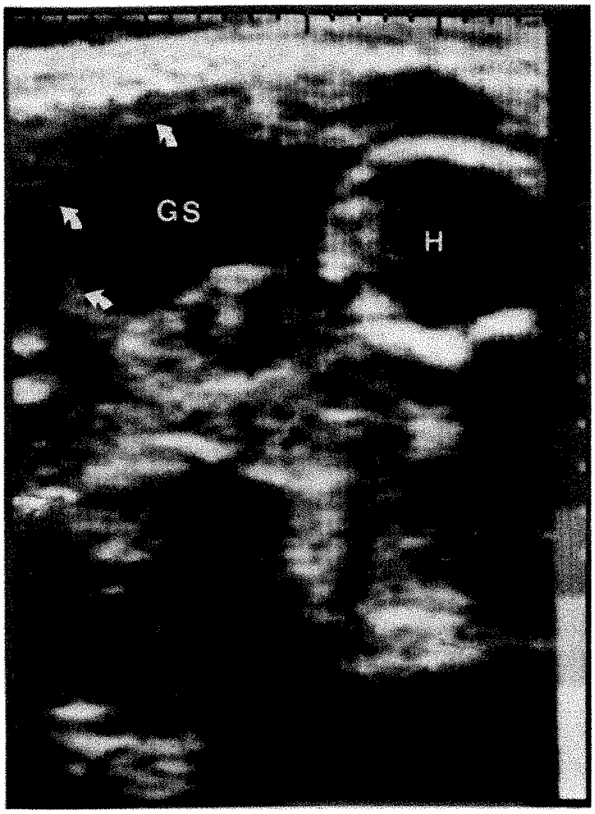

Fig. 9. A twin pregnancy is shown with one twin eventuating in a missed abortion and shown as a small gestational sac *(GS)*; *arrows* point to the thin wall of the sac simulating a meningocele adjacent to the fetal head of the other twin. See text for method of making correct diagnosis.

which a stunted gestational sac is situated next to the head of a normal fetus (Fig. 9). The sonographer should consider this rare possibility if a previous scan, obtained in the first trimester of pregnancy, showed twin gestational sacs. However, the diagnosis can only be made by carefully noticing that the cystic mass does not exhibit motion in conjunction with that of the fetus. Usually, the stunted sac is completely resorbed in the latter part of pregnancy.

On rare occasions, the nature of craniocervical masses may be difficult to ascertain. For instance, the sonographic appearance of conjoined twins may be mistaken for a cephalic mass.[5] Additionally, when a fetus is in the supine position, ultrasonic examination of the vertebral column is not easy, so that the relationship of the mass to the spine is not fully appreciated. Sometimes, the abnormality is just not ultrasonically apparent on the first examination, as in Case No. 1. Thus, the pregnant woman who is at high-risk for having a fetus with a craniospinal defect or other abnormality (because amniotic fluid AFP is high or the history is positive[6]) should be rescanned at weekly or biweekly intervals, depending on the clinical exigencies.

In conclusion, we believe that knowledge of the ultrasonic findings which characterize the various forms of fetal cranial or craniocervical masses may be helpful in allowing the sonographer to make the correct diagnosis. Furthermore, we recommend repeated ultrasonic examinations in those high-risk pregnancies in which an abnormality is either not seen or not clearly defined by the first study.

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Comparative newborn anthropometric data in symmetric versus asymmetric intrauterine growth retardation

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Autopsy studies have shown that the fetal liver is more severely affected than the brain in asymmetric fetal intrauterine growth retardation (IUGR). This "brain-sparing" concept has led to the suggestion that ultrasonic measurements of biparietal diameter (BPD) are of limited value in detecting IUGR since the BPD is an indirect reflection of head and brain size. This study compares the effects of asymmetric and symmetric IUGR on newborn anthropometric measurements, including head circumference (HC), birth weight (BW), length (L), and ponderal index (PI) in 33 growth-retarded infants divided into two groups, asymmetric (uteroplacental failure) and symmetric (maternal smoking, drug exposure, congenital anomalies, intrauterine infection), on the basis of the etiology of the IUGR. The percentage reduction in size from mean values for the same gestational age was calculated for each parameter. None of the newborn parameters were useful in distinguishing between infants with asymmetric and those with symmetric IUGR. Particularly noteworthy is the fact that the percentage decrease in HC was similar in both groups. Therefore, the concept of absolute "brain sparing" in asymmetric IUGR is erroneous, and ultrasonic measurements of BPD should be of value in detecting this form of IUGR. (AM. J. OBSTET. GYNECOL. 138:518, 1980.)

AUTOPSY studies[1, 2] in small-for-gestational-age (SGA) infants have revealed two basic patterns of IUGR (intrauterine growth retardation). One of these is designated "symmetric" IUGR because all body organs tend to be proportionately reduced in size. Etiologic factors responsible for symmetric growth retardation include intrauterine infections, such as rubella and cytomegalovirus, chromosome abnormalities, other congenital anomalies, maternal malnutrition, and smoking. Ultrasonically, the symmetric SGA fetus demonstrates a "reduced growth potential" pattern of biparietal diameter (BPD) growth which begins prior to the third trimester. Although BPD size increases from one examination to the next, absolute values consistently fall below −2 SD on the normal growth curve.[3, 4]

The other type of IUGR is "asymmetric" in nature, with some body organs more affected than others. For example, the fetal liver tends to be disproportionately small in comparison to the fetal brain.[1, 2] Asymmetric growth retardation is due to uteroplacental insufficiency and is commonly seen in pregnancies complicated by chronic hypertension, preeclampsia, and advanced diabetes. Ultrasonically, biparietal growth is essentially normal in these fetuses until some point in the third trimester, when abnormal slowing and eventual total arrest of BPD growth is observed.[3, 4]

Many studies have evaluated the reliability of ultrasonic measurements of BPD in detecting IUGR.[5-8] Accuracy rates from as low as 56% to as high as 93% have been reported. The reason for the high percentage of undetected cases of IUGR in some studies is unclear. One common explanation is that in asymmetrically growth-retarded fetuses the brain is preferentially spared at the expense of the body. Since the biparietal diameter is a reflection of head size and, indirectly, brain size, abnormal slowing of, or reduction in, BPD growth might not always occur in this form of IUGR.

This "brain sparing" theory is based upon experiments in rats, where ligation of the uterine artery that supplied one horn of a bicornuate uterus resulted

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Received for publication May 8, 1980.

Accepted July 17, 1980.

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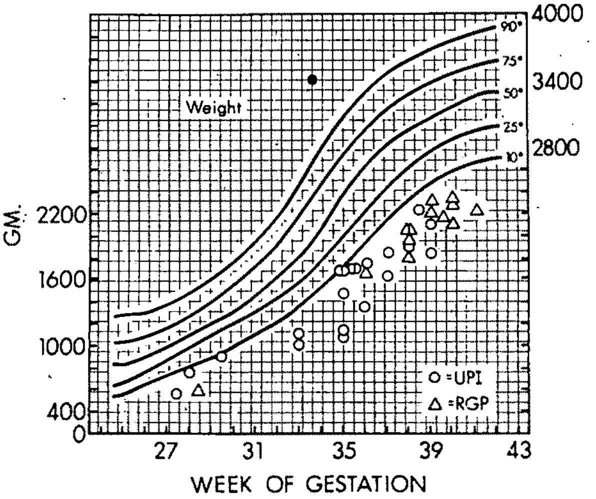

Fig. 1. Birth weights in 33 newborn infants with symmetric and asymmetric growth retardation (plotted on Colorado Intrauterine Growth Curve[15]). *UPI:* Uteroplacental insufficiency. *RGP:* Reduced growth potential.

Table I. Parameters of fetoplacental status in 20 SGA infants with uteroplacental insufficiency

Parameter	*No. of patients tested*	*Abnormal*	
		No.	*%*
Abnormal estriol pattern	12	8	67
Low human placental lactogen	10	10	100
Antepartum meconium	20	10	50
Positive contraction stress test +/or late deceleration in labor	19	18	95
Abnormal placental histologic features	8	6	75

in asymmetric fetal growth retardation in which the brain was minimally affected.[9] Acute ablation of the blood supply to a portion of the placenta has produced similar results in monkeys.[10] However, comparison of growth retardation in human fetuses with that in subhuman primate models may not be appropriate, since the brain of the rhesus monkey is more advanced in maturation and development than that of the human fetus. Nevertheless, this "brain sparing" concept has become quite popular in the obstetric literature[5, 11–14] and has led many investigators to suggest that ultrasonic cephalometry is of limited value in the detection of IUGR. The purpose of this study was to test this hypothesis by examining the effects of symmetric and asymmetric IUGR on head circumference as well as other anthropometric parameters in newborn infants.

Material and methods

The study population consisted of 33 consecutive SGA infants evaluated in the Perinatal Laboratory. Twenty of the infants suffered from asymmetric growth retardation due to uteroplacental insufficiency, as confirmed by multiple parameters of placental function (Table I). Complications of pregnancy in this group are listed in Table II. Thirteen infants were classified as symmetrically growth retarded on the basis of: (1) the existence of factors known to predispose to this form of IUGR (Table III), (2) the absence of fetal distress during labor, and (3) a "reduced growth potential" type of biparietal growth pattern on ultrasonography.

Table II. Predisposing factors in 20 infants with asymmetric IUGR

Factor	*No. of cases*
Chronic hypertension	6
Preeclampsia	11
Cardiac disease	1
Diabetes	1
Unknown	1
Total	20

Table III. Predisposing factors in 13 infants with symmetric IUGR

Factor	*No. of cases*
Maternal smoking	6
Drug addiction	1
Maternal leukemia and chemotherapy during pregnancy	1
Maternal red cell aplasia with severe anemia	1
Congenital cytomegalovirus infection	1
Multiple congenital anomalies	2
Trisomy 13	1
Total	13

Table IV. Comparative anthropometric data among infants with symmetric and those with asymmetric IUGR

Parameter	*Symmetric IUGR—13 infants (%)*	*Asymmetric IUGR—20 infants (%)*
Mean % decrease in:		
Birth weight	31	37
Body length*	6.8	10.6
Head circumference	6.3	8.5
Infants with ponderal index <10th centile	54	50

*Statistically significant, $p < 0.01$.

Newborn anthropometric measurements, including birth weight, body length, and head circumference values, as well as ponderal indices, were obtained on all infants and plotted by gestational age on Colorado Intrauterine Growth Curves.[15] The observed measurements were compared with mean values for the same gestational age, and the percentage difference was calculated for each parameter.

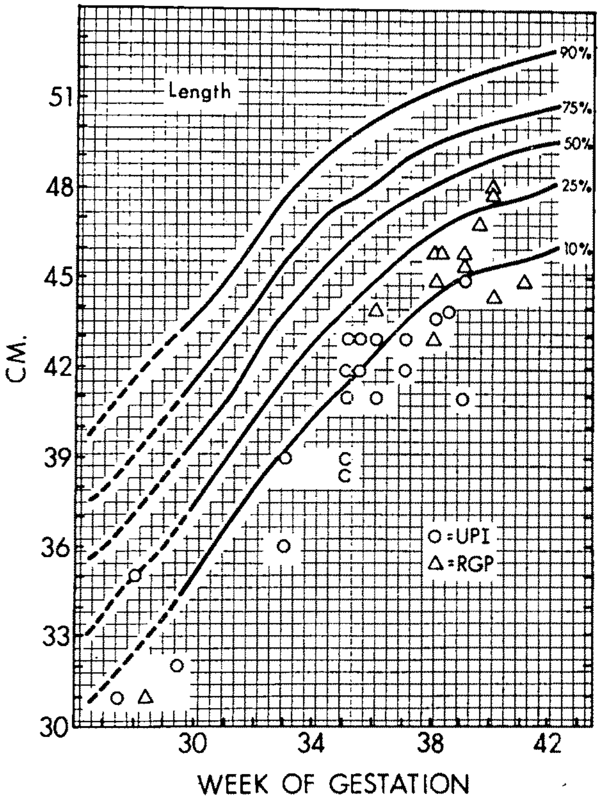

Fig. 2. Body lengths in 33 newborn infants with symmetric and asymmetric growth retardation (plotted on Colorado Intrauterine Growth Curve[15]).

Results

Body weight. All infants in both the symmetric and asymmetric groups plotted below the tenth percentile for gestational age (Fig. 1). The effect on body weight was not significantly different ($p = 0.10$) in symmetric versus asymmetric IUGR (Table IV). Among asymmetrically growth-retarded infants, body weight was reduced by an average of 37% when compared to mean values for infants of similar gestational age. The average reduction in body weight among symmetrically growth-retarded infants was 31%.

Body length. Fifteen (75%) of the 20 asymmetrically growth-retarded newborn infants plotted below the tenth percentile for gestational age, and the other five fell between the tenth and twenty-fifth percentiles (Fig. 2). The average reduction in body length as compared to AGA infants of similar gestational age was 10.6% (Table IV).

Six (46%) of the 13 symmetrically growth-retarded infants plotted below the tenth percentile (Fig. 2). Five fell between the tenth and twenty-fifth percentiles, and two between the twenty-fifth and fiftieth percentiles. The average reduction in body length compared to mean values for AGA singletons of similar gestational age was 6.8% (Table IV).

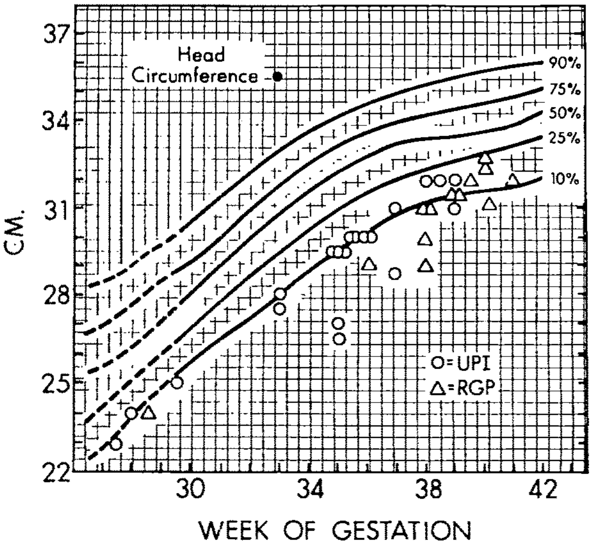

Fig. 3. Head circumference values in 33 newborn infants with symmetric and asymmetric growth retardation (plotted on Colorado Intrauterine Growth Curve[15]).

Head circumference. Fifteen (75%) of the 20 asymmetrically growth-retarded infants had head circumference values at or below the tenth percentile for gestational age (Fig. 3). The other five newborn infants plotted between the tenth and twenty-fifth percentiles. The average reduction in head circumference compared to mean values for AGA singletons matched for gestational age was 8.5%.

Head circumferences in nine (69%) of the 13 symmetrically growth-retarded newborn infants plotted at or below the tenth percentile, and four fell between the tenth and twenty-fifth percentiles (Fig. 3). There was an average decrease in head circumference of 6.3% below mean values for AGA infants of similar gestational age. When the symmetric and asymmetric SGA groups were compared, the percentage decrease in head circumference was not found to be significantly different ($p = 0.10$) (Table IV).

Ponderal index. Ten (50%) of the 20 asymmetrically growth-retarded infants had ponderal indices below the tenth percentile for gestational age (Fig. 4). Three infants plotted between the tenth and twenty-fifth percentiles, four fell between the twenty-fifth and fiftieth percentiles, and three plotted above the fiftieth percentile.

Ponderal indices below the tenth percentile for gestational age were observed in seven (54%) of the 13 infants with symmetric growth retardation (Fig. 4). Five infants were between the tenth and twenty-fifth percentiles, and one plotted at the fiftieth percentile.

Comment

None of the newborn anthropometric parameters measured in this study were useful in distinguishing between infants with symmetric and those with asymmetric IUGR. Approximately equal percentages of infants in each group had ponderal indices below the tenth percentile for gestational age, and the mean reductions in birth weight, body length, and head circumference were also similar (Table IV). Particularly noteworthy is the fact that the percentage decrease in head circumference was approximately 8.5% in asymmetrically growth-retarded newborn infants. Therefore, the concept of absolute "brain sparing" in asymmetric IUGR is erroneous. This finding is supported by the autopsy data of Naeye and co-workers,[1, 16, 17] who compared the brain weights of symmetric and asymmetric SGA neonates to those of AGA infants of similar gestational age. The mean reduction in brain weight among asymmetric SGA fetuses who suffered from uteroplacental insufficiency was 18%. Among symmetrically growth-retarded infants with rubella and Down's syndrome, the average decreases in brain weight were 21% and 19%, respectively. Although the absolute reductions in brain weight were similar in both forms of IUGR, other body organs did tend to be more severely affected in the asymmetric group. For example, asymmetric SGA infants demonstrated mean reductions in liver and spleen weights of 53% and 57%, respectively, as compared to only an 18% decrease in brain weight. In contrast, all body organs tended to be proportionately reduced in size in the symmetrically retarded infants. For example, among infants with congenital rubella, the mean reductions in brain, liver, and spleen weights were 21%, 23%, and 28%, respectively. Therefore, use of the terms "symmetric" and "asymmetric" is justified on this basis, but the concept of absolute "brain sparing" in asymmetric IUGR is incorrect and misleading.

Since brain size and head circumference are reduced in asymmetric IUGR, ultrasonic measurement of the biparietal diameter should be of value in the prenatal detection of this form of growth retardation. Indeed, the major problem with the use of biparietal measurements has been "overdiagnosis" and not "underdiagnosis" of IUGR.[18] This is because inadequate growth of the head from one examination to the next can be related to other factors, such as technical error in correctly identifying and measuring the true biparietal diameter. Another problem may be related to normal biologic variation in cranial morphology. The biparietal diameter reflects only one dimension of head size, so that less than average biparietal growth need not always correlate with IUGR. Various techniques have been proposed to overcome this problem, including ultrasonic measurement of fetal head circumference,[19] endocranial volume,[20] and ratio of head to abdominal circumference.[21, 22] The latter technique is based upon the fact that the liver is more severely affected than the brain in asymmetric IUGR. Consequently, the abdominal circumference measured at the level of the umbilical vein may be disproportionately small in comparison to fetal head circumference when true asymmetric IUGR exists.

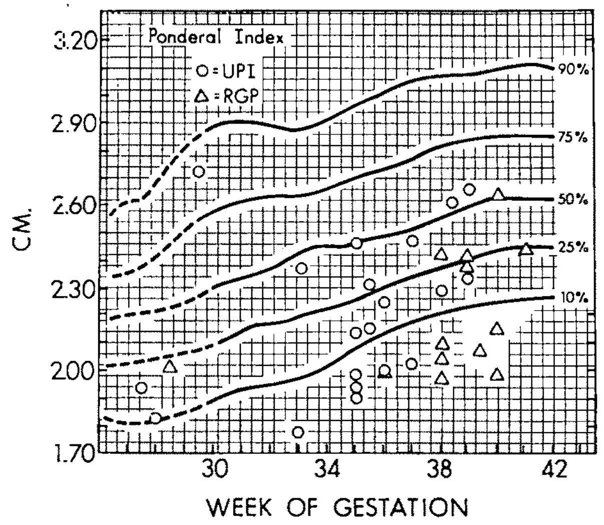

Fig. 4. Ponderal indices in 33 newborn infants with symmetric and asymmetric growth retardation (plotted on Colorado Intrauterine Growth Curve[15]).

In summary, the average decrease in brain size is approximately 18% in asymmetric IUGR. This is reflected by an average 8.5% decrease in fetal head circumference for gestational age. The fetal brain is not spared in asymmetric IUGR, and ultrasonic measurement of the biparietal diameter can be of value in detecting this form of IUGR.

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Blood anions and electrolytes during ritodrine infusion in preterm labor

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Seven patients with threatened premature labor and six nonpregnant female control subjects were studied for 12 to 24 hours during ritodrine infusion in order to re-evaluate the effects of this beta-adrenergic tocolytic agent on blood anions, electrolytes, and acid-base balance. Dexamethasone was given intramuscularly in all patients and three control subjects at the beginning of ritodrine infusion. Lactate, pyruvate, betahydroxybutyrate, sodium, potassium, chloride, bicarbonate, pH, glucose, and insulin levels were measured in blood samples obtained at intervals of 2 to 6 hours. While pH remained in the normal range, increases in lactate and pyruvate levels and decreases in bicarbonate values resulted in a significant increase in anion gap 4 to 6 hours after the beginning of treatment. The blood potassium level fell simultaneously. These effects were transient, lasting less than 10 hours, despite continuation of ritodrine infusion and maintenance of tocolysis and tachycardia. Although larger increases in the blood glucose level were observed with combined therapy in control subjects, dexamethasone had no additional influence on potassium concentrations. Monitoring of patients treated by intravenous ritodrine should include routine measurements of blood glucose and electrolyte levels in order to detect its potentially harmful metabolic side effects for mother and fetus. (AM. J. OBSTET. GYNECOL. 138:523, 1980.)

EFFECTIVENESS and adverse effects of betasympathomimetics such as terbutaline, salbutamol, fenoterol, and ritodrine, now widely used for inhibition of premature labor, have been the subject of extensive reviews in recent years.[1] In most patients, only cardiovascular side effects of these drugs are reported to cause mild discomfort.[2] However, life-threatening hemodynamic[3] and metabolic[4] disturbances have been occasionally induced by these agents in pregnant women.

Metabolic alterations caused by beta-adrenergic stimulation have been described mainly in obstetric conditions.[5] However, amplitude and duration of these alterations have not been fully characterized, so that guidelines for detection of potentially harmful complications of these drugs, such as hypokalemia or metabolic acidosis, are not available. In the current study, anions, electrolytes, and glucose and insulin levels were measured for periods up to 24 hours in pregnant patients and female nonpregnant control subjects who received a prolonged intravenous infusion of ritodrine, in order to delineate the plasma profiles of these blood constituents in routine clinical conditions.

Subjects and methods

Pregnant patients and normal nonpregnant control subjects gave their informed consent for participation in this study after full explanation of its aims and potential risks. Seven women admitted during the third trimester of gestation because of preterm labor and treated by intravenous infusion of ritodrine were investigated during the first 24-hour period after tocolytic therapy was begun. None of these patients had any other abnormality. Blood samples were obtained through an indwelling catheter inserted in a forearm vein for measurements of plasma levels of lactate, pyruvate, sodium, potassium, chloride, bicarbonate, betahydroxybutyrate, and pH. Blood glucose, insulin, and pyruvate levels in patients were not considered,

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This study was partially supported by the Ministère Belge de la Politique Scientifique (Actions de Recherche Concertées) and the Belgium F.N.R.S.

Received for publication February 1, 1980.

Revised July 15, 1980.

Accepted July 21, 1980.

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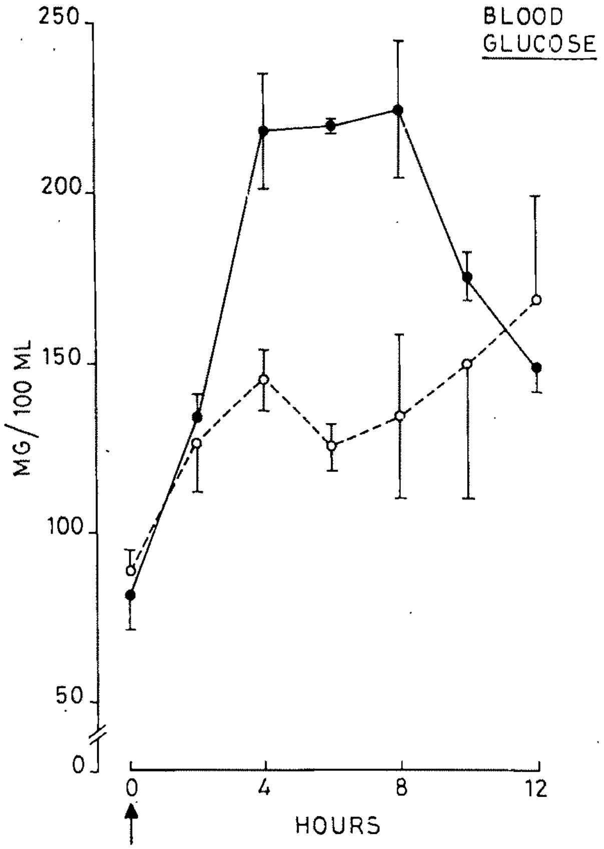

Fig. 1. Mean (±SEM) blood glucose values during treatment with intravenous ritodrine (six control subjects) with (—) or without (----) intramuscular dexamethasone. The arrow indicates the beginning of the treatment.

Table I. Blood values in patients during ritodrine infusion (n = 7)

	A	*B*	*p*
Potassium (mmoles/L)	3.5 ± 0.1	2.8 ± 0.1	<0.005
Bicarbonate (mmoles/L)	19.0 ± 0.9	13.7 ± 0.8	<0.01
Lactate (mmoles/L)	0.8 ± 0.1	4.9 ± 0.6	<0.001
Anion gap (mmoles/L)	5.0 ± 1.4	8.6 ± 0.9	NS
Betahydroxybutyrate (mmoles/L)	0.22 ± 0.05	0.29 ± 0.09	NS

A: Basal levels (mean ± SEM); B: maximal (minimal) values observed 2 to 12 hours after ritodrine infusion was begun (mean ± SEM). NS = Not significant.

Table II. Blood values in control subjects during ritodrine infusion

	A (*n* = 6)	B_1 (*n* = 3)	B_2 (*n* = 3)
Potassium (mmoles/L)	3.7 ± 0.1	2.7 ± 0.1	2.6 ± 0.1
Bicarbonate (mmoles/L)	20.8 ± 0.8	19.8 ± 0.7	15.3 ± 0.7
Lactate (mmoles/L)	1.0 ± 0.1	4.6 ± 1.1	6.8 ± 0.4
Pyruvate (mmoles/L)	0.11 ± 0.01	0.27 ± 0.03	0.53 ± 0.01
Lactate/pyruvate	8.9 ± 0.9	17.0 ± 2.3	12.9 ± 0.8
Anion gap (mmoles/L)	5.0 ± 1.1	7.5 ± 1.4	10.6 ± 1.9
pH	7.38 ± 0.01	7.36 ± 0.03	7.34 ± 0.02
Glucose (mg/100 ml)	86 ± 6	168 ± 30*	223 ± 20*
Insulin (μU/ml)	12 ± 3	67 ± 32	78 ± 28

A: Basal levels (mean ± SEM); B_1: maximal (minimal) levels observed during ritodrine alone (mean ± SEM); B_2: maximal (minimal) levels observed during ritodrine and dexamethasone (mean ± SEM).

*B_1 versus B_2: $p < 0.001$.

except for the basal blood glucose value, which was in the normal range for all subjects.

After a basal blood sample had been collected, ritodrine diluted in a 5% dextrose solution was infused by a pump at a rate increasing from 50 up to 150 μg/min until the tocolytic effect was obtained; blood samples were drawn at 2, 4, 6, 12, 18, and 24 hours. At 0 and 12 hours 12 mg of dexamethasone was injected intramuscularly in order to accelerate fetal lung maturation. Intravenous potassium supplements (40 mmoles/500 ml of dextrose solution) were added when the plasma potassium value was lower than 3.0 mmoles/L.

In six nonpregnant members of the medical staff, aged 22 to 29, ritodrine diluted in saline solution was infused at a constant rate (150 μg/min). Three subjects received 12 mg of dexamethasone intramuscularly at the beginning of the ritodrine infusion. Blood was drawn at 2-hour intervals for 12 hours for determination of plasma lactate, pyruvate, sodium, potassium, chloride, bicarbonate, pH, glucose, and insulin values. Lactate and pyruvate were assayed by the method of Henry and associates.[6] Electrolytes were determined by emission flame photometry; plasma glucose was measured on a Beeckman glucose analyzer (glucose oxidase method). Insulin was assayed by radioimmunoassay; pH was measured on a radiometer M 27 pH meter, and betahydroxybutyrate was measured fluorimetrically. The anion gap was estimated as (Na + K + Ca) − (Cl + HCO_3 + protein).[7] Significance was determined by means of Student's t test for paired observations; p values of <0.05 were regarded as significant.

Results

Serum potassium fell significantly in patients (Table I) as well as in normal control subjects (Table II), with minimal values reached in both groups 4 hours after the ritodrine infusion was begun. Control subjects not receiving a supplement exhibited a return to normokalemia 10 to 12 hours after the treatment was started.

All other metabolic effects in the control group were clearly observed with ritodrine alone but appeared more marked with the combined administration of ritodrine and dexamethasone (Table II). However,

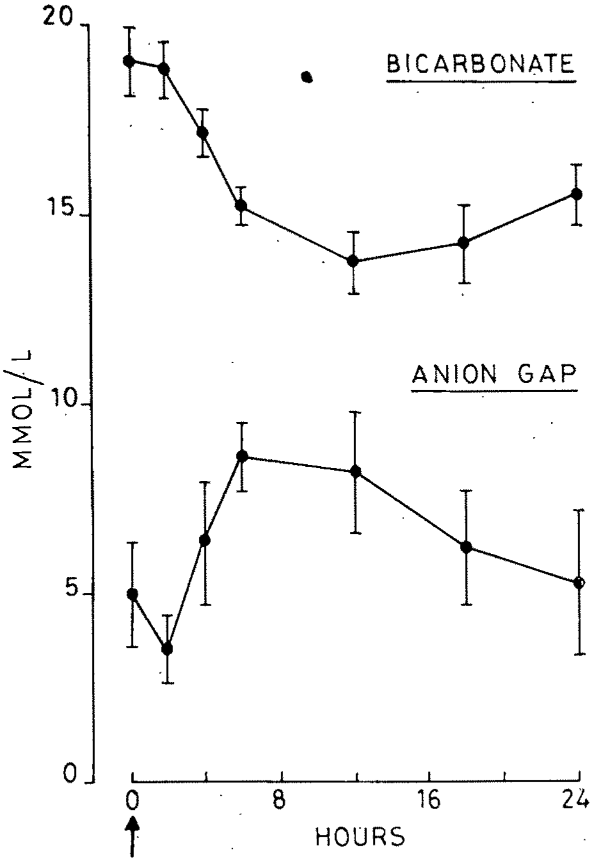

Fig. 2. Mean (±SEM) plasma bicarbonate and anion gap values during treatment with intravenous ritodrine (seven patients). The arrow indicates the beginning of the treatment.

since only three subjects were investigated in each subgroup, these differences were not significant, except for blood glucose levels, which were markedly higher ($p < 0.001$) in subjects receiving dexamethasone (Fig. 1). Plasma sodium and chloride concentrations (ranging from 130 to 141 mmoles/L and from 98 to 111 mmoles/L, respectively) were not affected significantly during ritodrine infusion. Plasma bicarbonate concentration decreased in patients (Table I) and control subjects (Table II), with minimal values being observed 12 and 8 hours, respectively, after the beginning of treatment (Fig. 2).

A significant rise of plasma lactate was detected after 2 hours of infusion in patients (0.8 ± 0.1 versus 2.0 ± 0.2 mmoles/L, $p < 0.02$) and control subjects (1.0 ± 0.1 versus 2.5 ± 0.2 mmoles/L, $p < 0.001$), while maximal concentrations of lactate (Table I and II) occurred after 6 and 8 hours, respectively (Fig. 3).

Pyruvate concentrations (Table II), measured only in normal control subjects, increased during ritodrine, with maximal values observed 8 hours after treatment was begun (Fig. 3).

The lactate/pyruvate ratio (Table II) showed an abrupt increase 2 hours after the ritodrine infusion was begun; this increase was maintained subsequently above 12 up to 12 hours of infusion.

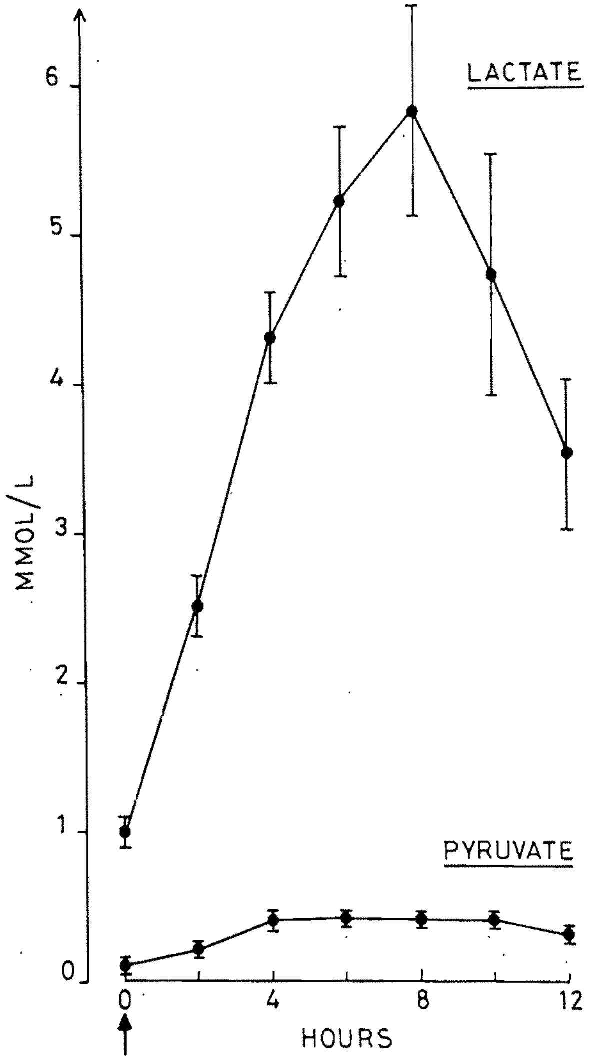

Fig. 3. Mean (±SEM) plasma lactate and pyruvate values during treatment with intravenous ritodrine (six control subjects). The arrow indicates the beginning of the treatment.

In some individuals, an extreme rise in lactate and a fall in bicarbonate levels were associated with an anion gap above 10 mmoles/L. However, anion gap increase reached significance only in the group of control subjects as a whole (Fig. 2). A systematic control of pH was performed only in control subjects; a significant decrease was observed, but the values remained in the normal range. Measurements of betahydroxybutyrate levels in five patients did not disclose any effect of ritodrine infusion (Table I).

Comment

The most striking metabolic effects in this series (hyperlactacidemia and hypokalemia) were observed in pregnant subjects and nonpregnant control subjects, suggesting that pregnancy itself does not predispose to these effects.

Hyperlactacidemia has been described in previous reports,[8] but the course of lactate variations in plasma during a sustained infusion had not yet been fully characterized. The increased production of lactate caused by beta-adrenergic stimulation is thought to be due to the combination of increased glycogenolysis and lipolysis[9]; beta-adrenergic stimulation causes enhanced glycogenolysis and glycolysis and results in the exaggerated production of lactate and pyruvate.

On the other hand, accelerated lipolysis provides large amounts of free fatty acids,[10] which have been shown to inhibit pyruvate oxidation and to favor anaerobic glycolysis and lactate accumulation and to increase the lactate/pyruvate ratio. [11] We observed such an elevation of the lactate/pyruvate ratio (Fig. 3) but we did not measure free fatty acid levels; lipolysis, if present, did not result in a significant elevation of production of ketone bodies since betahydroxybutyrate levels remained in the normal range in all subjects (Table I) and were not affected by prolonged infusion. The variations of these anions (lactate, pyruvate, and betahydroxybutyrate) in plasma during ritodrine infusion suggest that lactate was the major metabolic anion responsible for anion gap increase. We were not able to detect deviations of the blood pH from the normal range in our series. However, metabolic acidosis has been encountered occasionally in otherwise normal pregnant women undergoing acute tocolysis; in one single case, this acidosis has been clearly ascribed to an exaggeration of the hyperlactacidemia.[12]

Hypokalemia resulting from administration of beta-adrenergic agents has been frequently reported in clinical series.[13] This effect has been regarded as a by-product of the combined glucose and insulin elevations which induce accelerated transfer of potassium into the intracellular compartment.[14] We did not observe significant differences in the amplitude of the plasma potassium drop in normal control subjects, who had mild hyperglycemia (168 ± 30 mg/dl), or in control subjects receiving dexamethasone, who exhibited much higher blood glucose levels (223 ± 20 mg/dl).

Another mechanism for intracellular transfer of potassium could be found in the ultimate action of beta-adrenergic agents on smooth muscles[15]: Beta-receptor agonists induce relaxation of smooth muscle by stimulating adenylate cyclase which in turn produces cyclic adenosine monophosphate, activation of the Na^+/K^+ pump, and net exchange of intracellular Na^+ for extracellular K^+. The increased gradient for Na^+ across the cell membrane accelerates the rate of Na^+/Ca^{++} exchange, resulting in increased Ca^{++} efflux from the cytoplasm and finally changes in intracellular Ca^{++} distribution, which decreases the muscle contractibility. A byproduct of these movements of cations across the cell membrane could be a net flux of potassium from the extracellular to the intracellular compartment, with significant hypokalemia.

The return to normal of most metabolic disturbances induced by ritodrine could be related to the selective desensitization of beta-adrenergic receptors, which has been characterized in various systems in vitro and in vivo.[16] The decrease in the number of binding sites for beta-adrenergic agonists observed after exposure to isoproterenol probably results in loss of cell responsiveness. The maintenance of tachycardia and tocolysis for days in pregnant women given betamimetics while metabolic effects, even for submaximal doses, are transient in terms of hours could reflect differential desensitization processes in various target organs, with liver and skeletal muscles exhibiting a faster and more pronounced loss of beta-adrenergic responsiveness than the heart and uterus.

Although the number of subjects receiving ritodrine alone was small, our data suggest that most metabolic disturbances induced by betamimetics, and particularly blood glucose variations, are exaggerated when large doses of corticosteroids are associated with tocolytics in order to foster fetal lung maturation.

From a clinical point of view, metabolic monitoring of all pregnant patients given intensive beta-adrenergic therapy for tocolysis should include determinations of blood glucose and electrolytes at intervals of 2 to 6 hours during the first day of betamimetic treatment. Moreover, particular caution must be recommended in high-risk patients such as those with diabetes and those receiving diuretics.

We thank A. Elslander, R. N., for her valuable help in this study.

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Brain uptake of meperidine in the fetal lamb

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The uptake of meperidine by the brain of the fetal lamb was investigated by determining the concentration of meperidine in the fetal brachiocephalic artery and sagittal vein after intravenous and intramuscular administration to the mother. A positive arteriovenous gradient across the fetal brain existed for 10 minutes after intravenous administration, and for 20 to 25 minutes after intramuscular administration, thus indicating the uptake of meperidine by the fetal brain. The peak concentration of unbound meperidine in the fetal brain, as estimated from the plasma concentration of free meperidine at equilibrium, was three to four times greater after intravenous administration than after intramuscular administration. The findings suggest that, during labor, the route of administration of meperidine will determine the time course of meperidine in the fetal brain and, consequently, the time action of effects seen in the neonate. The lag time required for plasma-brain equilibration may explain the lack of correlation between the incidence of neonatal respiratory depression and the levels of meperidine in the cord after intramuscular administration.
(AM. J. OBSTET. GYNECOL. 138:528, 1980.)

SHORT-TERM administration of meperidine to the mother during labor has been reported to result in an increased incidence of neonatal respiratory depression. Furthermore, it appears that the incidence of neonatal respiratory depression may be related to the time of administration of meperidine during labor. In the most comprehensive retrospective study comprising 650 patients, Shnider and Moya[1] reported that, when meperidine was administered intramuscularly within 1 hour of delivery, the incidence of depressed babies was not significantly different from that in the control series. However, a significant increase in the incidence of depressed babies was observed in the second hour after the administration of 50 mg of meperidine and in the second and third hours after the administration of 75 or 100 mg of meperidine.

Several attempts have failed to demonstrate a correlation between the incidence of respiratory depression and the levels of meperidine in cord blood.[2-6] Morrison and associates[2, 6] attempted to correlate the incidence of depression with the levels in cord blood of nor meperidine, an N-demethylated metabolite of meperidine. However, since their studies were carried out with the use of the nonspecific methyl-orange complex method, it is not possible to form any conclusion in

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Supported in part by National Institute on Drug Abuse Grants Nos. DA-01825 and DA-01707, National Institute of Child Health and Human Development Grant No. HD-00213, and National Cancer Institute Core Grant No. CA-08748.

Preliminary results were presented at the International Narcotic Research Conference, 1978, and the New England Perinatal Society Meeting, 1979.

Received for publication October 26, 1979.

Revised July 17, 1980.

Accepted July 22, 1980.

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**Recipient of a Faculty Development Award in Pharmacology from the Pharmaceutical Manufacturers' Association Foundation.*

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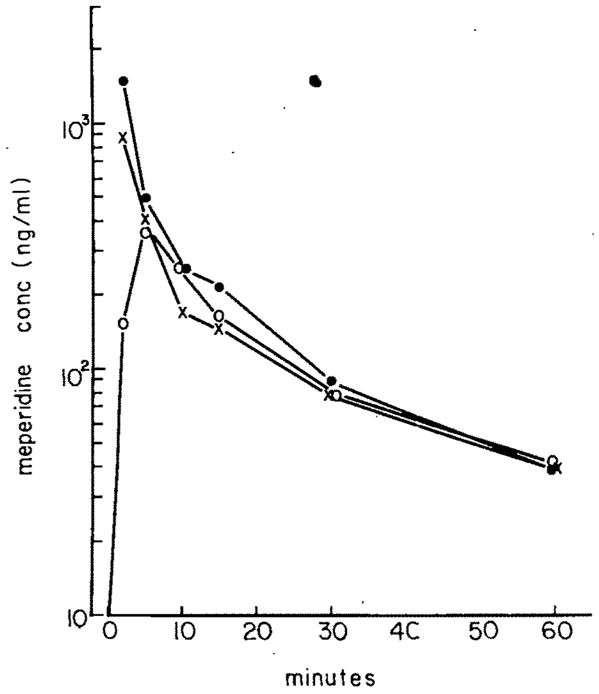

Fig. 1. An example of the time course of meperidine in the maternal aorta (•—•), fetal brachiocephalic artery (x - x), and fetal sagittal vein (o—o) after intravenous administration of meperidine (2.5 mg/kg) to the mother. (From Szeto and associates,[8] by permission of Elsevier/North Holland Biomedical Press.)

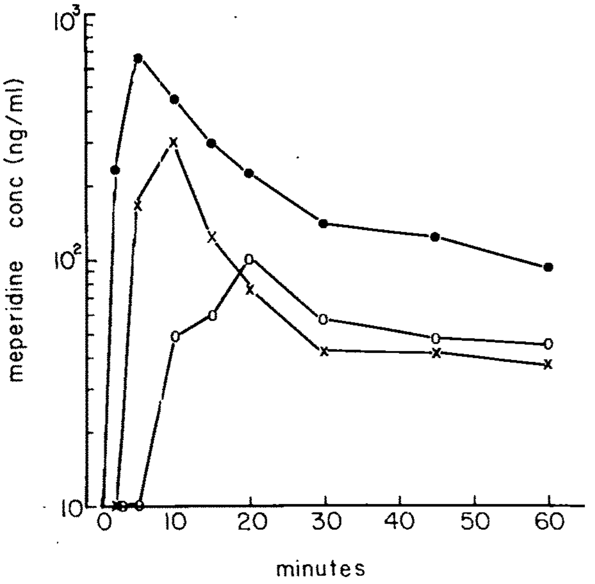

Fig. 2. An example of the time course of meperidine in the maternal aorta (•-•), fetal brachiocephalic artery (x - x), and fetal sagittal vein (o-o) after intramuscular administration of meperidine (2.5 mg/kg) to the mother. (From Szeto and associates,[8] by permission of Elsevier/North Holland Biomedical Press.)

regard to the role of this metabolite in respiratory depression. More recently, Kuhnert and associates,[7] by means of a gas chromatography-mass spectrometry method, found no relationship between the drug-delivery interval and the concentration of normeperidine in the umbilical cord.

Meperidine is a narcotic analgesic which depresses respiration by virtue of a direct effect on the brain stem respiratory centers. Therefore, one can expect that the time course of respiratory depression will be better related to the time course of drug concentration in the central nervous system rather than in plasma. The purpose of this study was to investigate the rate of uptake of meperidine by the fetal brain as related to the time course of meperidine in fetal plasma. Since such a study could not be performed in the human fetus, the chronic pregnant ewe model was used. Levels of meperidine in the brain were not determined because a large number of animals would have been necessary for the generation of a time curve. The same information was obtained by a determination of the arteriovenous concentration difference of meperidine across the fetal brain as a function of time after drug administration. Preliminary results of this study were published in the Proceedings of the 1978 International Narcotic Research Conference.[8] The results are discussed here in more detail.

Material and methods

Animal preparation. Five pregnant ewes of mixed breed at 116 to 137 days' gestation (term is approximately 145 days) were used in this study. The surgical procedures were performed after the intrathecal administration of 1% lidocaine, which was supplemented intermittently with small intravenous doses of pentobarbital sodium, as necessary.

With the ewe in the left lateral position, a midline incision was made in the lower abdomen, and the uterus and its contents were delivered outside the abdominal cavity. A uterine incision was made over the fetal cranial vault and through the fetal scalp to expose the skull. A polyvinyl catheter was then introduced into the transverse sinus via the sagittal sinus, with the catheter tip lying 2 to 5 mm beyond the confluence of the sinuses. Dental acrylic was used to secure the catheter. Another catheter was introduced into the brachiocephalic artery via the axillary artery. One gram of ampicillin was placed into the amniotic cavity and the

Table I. Estimated concentration of free unbound meperidine in the fetal brain at equilibrium (n = 5)

Mode of administration	*Time for A-V equilibration (min)*	$C_{brain,\,eq}$ *(ng/ml ± SE)*
Intravenous	10	233 ± 22.9
Intramuscular	20-25	68 ± 2.1

From Szeto and associates,[8] by permission of Elsevier/North Holland Biomedical Press.

maternal abdominal cavity. Catheters were also placed in the maternal aorta and vena cava. All catheters were tunneled subcutaneously to the maternal flank and stored in a pouch.

Drug studies. A period of recovery from the surgical procedures of from 24 to 48 hours was allowed before any experiment was conducted. The ewe was allowed to stand unrestrained in an experimental cart, and had free access to food and water throughout the course of the study. After a control period of 30 to 60 minutes, samples of blood were collected from mother and fetus for the determination of blood gases and pH (Radiometer BMS3 Mk2 Blood Micro System). Only animals with blood gas values within the normal range were included in the study. Meperidine hydrochloride was then administered either intravenously or intramuscularly to the mother at a dose of 2.5 mg/kg. Samples of blood were collected from the maternal aorta, fetal brachiocephalic artery, and the fetal sagittal vein at 0, 2, 5, 10, 15, 20, 30, 45, and 60 minutes after administration of the drug. Maternal and fetal blood gases were again determined at 60 minutes after administration of the drug.

Analytical method. The concentration of meperidine in biofluid samples was determined by means of a combination of solvent extraction and gas-liquid chromatography.[9]

Results

All animals tolerated this dose of meperidine well, with no overt signs of toxicity. Maternal and fetal blood gases and pH values at 60 minutes after administration of meperidine were not significantly different from the predrug values. Of the five fetuses that were studied, two were killed in order to confirm the placement of the catheters. The other three fetuses were delivered vaginally at term and did well postnatally.

The meperidine plasma decay curves obtained in the five animals after an intravenous dose of meperidine were very similar. An example of the concentration of meperidine in the maternal aorta, fetal brachiocephalic artery, and fetal sagittal vein in one animal is shown in Fig. 1. In all five animals, maternal plasma levels declined in a biexponential fashion, and the mean apparent half life of the elimination phase was of the order of 20 minutes. Meperidine was transferred rapidly to the fetus, and the peak level in the fetal brachiocephalic artery occurred within 2 minutes after administration of the drug. The time course of meperidine concentration in the fetal brachiocephalic artery was similar to that in the maternal aorta. On the other hand, meperidine concentration in the fetal sagittal vein was initially significantly lower than that in the brachiocephalic artery. The level of meperidine in the sagittal vein rose rapidly and approached that in the brachiocephalic artery between 5 and 10 minutes in the five animals. Thereafter, the levels of meperidine in the sagittal vein remained slightly higher than those in the brachiocephalic artery, and declined at the same rate.

Fig. 2 shows an example of the levels of meperidine in the maternal aorta, fetal brachiocephalic artery, and sagittal vein after intramuscular administration to the mother. Maternal plasma levels rose rapidly and peaked between 5 and 10 minutes after administration of the drug. Meperidine was detectable in the fetal brachiocephalic artery within 5 minutes, and peaked at approximately 10 minutes, after administration of the drug. Fetal plasma levels remained consistently lower than maternal levels at all times. Meperidine was not detectable in the sagittal vein until 10 minutes, and did not peak until 20 minutes, after administration of the drug. Arteriovenous equilibration in the five animals took place between 20 and 25 minutes. After arteriovenous equilibration, venous levels remained slightly higher than arterial levels, and both declined at the same rate. The elimination half life of meperidine after intramuscular administration was more prolonged than that after intravenous administration, which suggests that the drug was still being absorbed up to 60 minutes after it had been administered.

Comment

As reported here, and observed in previous studies,[10] meperidine is rapidly transferred from mother to fetus after intravenous administration. The high concentration of drug in the fetal brachiocephalic artery which results from the bolus injection enters the fetal brain and can be distributed throughout brain tissue. Initially the concentration of meperidine in the blood entering the brain (brachiocephalic artery) is much greater than the concentration in the venous outflow from the brain (sagittal vein). This large arteriovenous (A-V) difference indicates rapid uptake of meperidine by brain tissue. As the concentration of meperidine in brain tissue increases, the clearance of the drug by the brain decreases. Uptake of the drug by the brain continues until a point in time when the concentration of it entering the brain equals the concentration of it emerging from the brain, and the clearance by the brain becomes zero. This was demonstrated to occur at approximately 10 minutes in the five animals after intravenous administration to the mother (Table I). At this point of A-V equilibration, the concentrations of free unbound drug in the brachiocephalic artery, sagittal vein, and the brain should be equal.[11] The concentration of meperidine in the brain would have reached a peak level at this time. Since the binding of meperidine to fetal plasma proteins is approximately 28%,[10] the concentration of free meperidine in the fetal brain at equilibrium ($C_{\text{brain eq}}$) can be estimated from the free drug concentration in plasma at the time of A-V equilibration. The fetal arterial and venous plasma level curves were extrapolated to the point at which they crossed, and the mean plasma level at the time of A-V equilibration was found to be 233 ± 22.9 (SE) ng/ml for the five animals (Table I). The total amount of meperidine that is actually distributed into the fetal brain tissue depends on the partition coefficient of meperidine between blood and brain tissue. Unfortunately, the percentage binding of meperidine to fetal brain tissue is not known at this time.

When the input process is completed, i.e., after A-V equilibration, the clearance of the brain actually becomes negative. As meperidine is being eliminated from the other parts of the body, the concentration of it entering the circulation to the brain begins to fall. The drug partitions back from brain tissue into blood, and the concentration in the venous outflow is higher than that in the inflow blood. The rate at which the drug is removed from the brain is ultimately determined by the overall rate of its elimination from the entire maternal-fetal unit.

When the same dose of meperidine is administered intramuscularly to the mother, both the rate and extent of distribution of the drug to the fetus are decreased in comparison to the values with intravenous administration (Figs. 1 and 2).[10] The plasma concentration of meperidine in the fetus is significantly lower after intramuscular administration than after intravenous administration. This may be explained by a significantly smaller concentration gradient across the placenta after intramuscular administration. In human neonates, Crawford and Rudofsky[5] measured the amount of meperidine and metabolites excreted into neonatal urine after intravenous and intramuscular administration to the mother, and found that significantly more drug and metabolites were recovered from neonates whose mothers had received meperidine intravenously. These observations suggest that more maternally administered drug is distributed to the fetus after intravenous than after intramuscular administration.

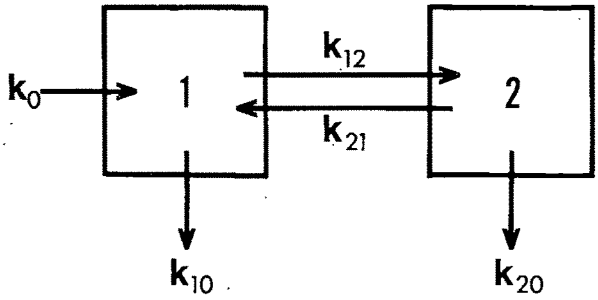

Fig. 3. Diagram of a two-compartment open model describing the disposition of meperidine in the fetal lamb, where *1* represents the central compartment, and *2* represents the peripheral compartment. k_0 is the rate of infusion of meperidine, k_{12} and k_{21} are the rate constants of transfer of meperidine between the central compartment and the peripheral compartment, k_{10} is the rate constant of elimination from the central compartment, and k_{20} is the rate constant of elimination from the peripheral compartment.

The uptake of meperidine by the fetal brain after intramuscular administration to the mother is demonstrated by the initial A-V difference of meperidine across the fetal brain (see Fig. 2). The initial uptake is significantly slower than with intravenous administration. This can be explained by the much lower concentration of meperidine in the brachiocephalic artery with intramuscular than with intravenous administration. A-V equilibration does not occur until 20 to 25 minutes after administration of the drug (Table I), which suggests that the peak concentration in the brain occurs significantly later with intramuscular administration. The concentration of free meperidine in the fetal brain at the point of A-V equilibration averages 68 ± 2.1(SE) ng/ml, which is three to four times lower than that achieved with intravenous administration. Therefore, it appears that the extent of exposure of the fetal brain to meperidine is also decreased with intramuscular administration because of the lower concentration of the drug in the brachiocephalic artery immediately after administration.

It was previously shown that the plasma decay curve of meperidine in the pregnant ewe and the fetus after intravenous administration was best fit by a biexponential equation with a rapid distribution phase and a slower elimination phase.[10] In pharmacokinetic terms, the disposition of meperidine in the pregnant ewe and the fetus can each be described by a two-compartment open model, with a central compartment that represents the rapidly perfused tissues and a peripheral compartment that represents the more slowly perfused tissues (Fig. 3). If one assumes that the peak pharmacologic effect is a function of peak drug level at the site of action, then the time of peak effect after administration of meperidine should be related to the time of peak level of meperidine in the central nervous system. Because of the rapid flow of blood to the brain, the central nervous system is generally assumed to be part of the central compartment. Therefore, it has been customary to relate the time course of respiratory depression to the time course of plasma levels of meperidine. The findings of this study, however, clearly demonstrate that there is a lag time between peak plasma level and peak brain level of meperidine in the fetus. The data suggest that the fetal brain should be considered to be part of the peripheral compartment and that the effect of meperidine on fetal respiration may correspond more closely to the time course of meperidine in the peripheral compartment. Similar observations have been reported by Kaiko and associates.[12] on the time course of effects of morphine in the human. The authors found that the time of peak changes in respiration, miosis, analgesia, and mood after administration of morphine corresponded most closely to the time course of levels of morphine in cerebrospinal fluid, which, in turn, could be shown to approximate the time course of morphine in the peripheral compartment.

This lag time that is necessary for plasma-brain equilibration may provide an explanation for the lack of correlation between the incidence of neonatal respiratory depression and the levels of meperidine in the cord. If delivery occurs soon after intramuscular administration of meperidine to the mother, the levels of meperidine in the cord may be quite high, yet the levels in the brain are still low (see Fig. 2). On the other hand, if delivery occurs after plasma-brain equilibration has taken place, the levels of meperidine in the cord may appear to be lower than at the earlier times, but the concentration of the drug in the brain may be at its peak. Furthermore, if the fetus is delivered when the concentration of meperidine in plasma is still declining rapidly because of distribution to the tissues, the drug will move rapidly out of the brain to maintain equilibrium with the more slowly perfused tissues, such as muscle and adipose tissues. In contrast, if delivery occurs after distribution of the drug to the tissues is nearly complete, the concentration of the drug in the brain will fall at the same rate as the concentration in plasma, and this will be governed primarily by the rate of metabolism and renal excretion.

That the actual time for plasma-brain equilibration obtained in the fetal lamb cannot be directly applied to the human fetus must be emphasized. We previously demonstrated that significant quantitative differences exist between the pharmacokinetics of meperidine in pregnant ewes and that in the human.[10] The rate of absorption of the drug after intramuscular administration is more rapid in the ewe than in the human. Peak levels of the drug in plasma are attained by 10 minutes after administration in the pregnant ewe but not until 1 hour in the human.[13] The mean apparent half life of meperidine in the pregnant ewe (20 minutes)[10] is significantly shorter than that in pregnant women (3.7 hours)[14] or nonpregnant human volunteers (3.2[15] and 3.7[16] hours). The slower rate of absorption and elimination of meperidine in the human should necessitate a longer time for plasma-brain equilibration of meperidine in the fetus. It is possible that the peak concentration of meperidine in the brain is not reached in the human fetus until 1 to 2 hours after intramuscular administration of the drug to the mother. This may explain the higher incidence of respiratory depression seen in neonates delivered 2 hours after intramuscular administration of meperidine to the mother.

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Early steps in prostaglandin metabolism in the human placenta

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The activities of the enzymes catalyzing the early steps in prostaglandin metabolism (the nicotinamide adenine dinucleotide [NAD]–linked 15-hydroxyprostaglandin dehydrogenase, the nicotinamide adenine dinucleotide phosphate [NADP]–linked 15-hydroxyprostaglandin dehydrogenase, and the 15-ketoprostaglandin Δ^{13} reductase) were measured in homogenates of term placenta. The NAD-linked enzyme possesses the highest activity of the three. The NADP-linked enzyme has much lower activity and probably acts physiologically as a 9-ketoprostaglandin reductase rather than as a 15-hydroxyprostaglandin dehydrogenase. Placental homogenates contain a low-molecular-weight, heat-stable inhibitor of the NADP-linked enzyme. The reaction catalyzed by the 15-ketoprostaglandin Δ^{13} reductase is the rate-limiting step. Analysis of the activity of these enzymes has shown that there is no significant relationship between them; furthermore, whether the placentas are from spontaneous single or twin deliveries, induced deliveries, or cesarean sections (in labor or not in labor) does not have a significant effect on the activity of the NAD- or NADP-linked dehydrogenases. (AM. J. OBSTET. GYNECOL. 138:534, 1980.)

FOLLOWING the observation that human placenta is an extremely rich source of a nicotinamide adenine dinucleotide (NAD)–linked 15-hydroxyprostaglandin dehydrogenase,[1] a systematic study was undertaken of the enzymes which catalyze the early steps in prostaglandin metabolism in the human placenta. These steps are shown in Fig. 1. The enzymes catalyzing these steps have been individually isolated, purified (usually to apparent homogeneity), and characterized.[2–5] An endogenous inhibitor of one of these enzymes, the nicotinamide adenine dinucleotide phosphate (NADP)-linked 15-hydroxyprostaglandin dehydrogenase, also has been identified and partially characterized.[5]

The present study compares the activities of these enzymes in individual placentas in order to determine which of the early steps of prostaglandin metabolism is rate limiting, to analyze whether there is a relationship in a given placenta between the activities of these enzymes or between the activity of the NADP-linked 15-hydroxyprostaglandin dehydrogenase and its inhibitor, and to evaluate whether the activities of these enzymes differ according to the nature of the delivery.

Material and methods

The placentas were obtained at term from spontaneous single or twin deliveries, induced deliveries, or cesarean sections (in labor or not in labor). None of the mothers had pre-eclampsia or eclampsia, but several required insulin because they were diabetic. All of the placentas were chilled immediately after delivery. Within 4 hours of delivery the villous tissue had been dissected from the membranes, rinsed in cold tap water, and homogenized. Portions of 75 to 110 grams were homogenized at top speed in a Waring Blendor with two volumes of buffer containing 5 mM potassium phosphate and 1 mM ethylenediaminetetraacetate (pH 7.0) The placentas were all used as the source of one or more of the prostaglandin-metabolizing enzymes or of the inhibitor of the NADP-linked 15-hydroxyprostaglandin dehydrogenase. Depending on the enzyme or enzymes to be isolated, 20% glycerol (v/v) was present or absent from the homogenization buffer. The NAD-linked 15-hydroxyprostaglandin dehydrogenase is quite labile but is stabilized by glycerol.[1] Consequently, 20% glycerol was generally present in

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This study was supported by grants from the United States Public Health Service (HD-07045) and from the Louis Block Foundation.

Received for publication November 9, 1979.

Revised May 21, 1980.

Accepted July 14, 1980.

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Table I. NAD-linked 15-hydroxyprostaglandin dehydrogenase activity in centrifuged placental homogenates*

Assay conditions	*Type of delivery and number of placentas*	*Units/placenta* $(\times 10^3)$	*Units/gm tissue* $(\times 10^3)$
1. Phosphate buffer pH 7.0 PGE_1	Single, spontaneous (8)	22,500 (±10,600)†	82.5 (±27.8)†
	Twins, spontaneous (1 pair)	7,000	28.6
		10,900	43.5
	Cesarean section, in labor (1)	14,800	58.2
	Cesarean section, not in labor (1)	30,000	96.6
	Induced (1)	11,600	36.4
2. Phosphate buffer pH 7.0 PGE_2	Single, spontaneous (20)	20,600 (±12,500)	58.6 (±27.9)
3. Phosphate buffer pH 7.0 PGE_1	Single, spontaneous (16)	8,640 (±6,020)	22.4 (±14.7)
	Cesarean section, in labor (1)	11,900	33.5
	Cesarean section, not in labor (3)	3,590	5.31
		5,210	6.28
		8,720	21.5
	Induced (2)	4,400	14.7
		9,860	37.2
4. Tris buffer pH 9.0 PGE_1	Single, spontaneous, normal (63)	44,400 (±24,600)	137 (±65)
	Single, spontaneous, diabetic (1)	77,300	226
	Single, spontaneous, heroin addict (1)	17,000	60.1
	Twins, spontaneous (1 pair)	27,500	104
		42,000	159
	Cesarean section, in labor (5)	21,800	81.6
		27,500	58.5
		28,500	92.5
		39,600	116
		53,100	125
	Cesarean section, not in labor (7)	22,200	74.9
		37,200	113
		40,100	157
		40,100	95.7
		59,900	126
		64,300	218
		69,100	270
	Induced (2)	15,500	43.4
		55,100	162

*The data for this table were obtained from enzyme preparations done in collaboration with Drs. S. Braithwaite and C. Westbrook and Ms. B. Lin. The buffer used to homogenize the placentas in preparation 3 did not contain glycerol; the other placentas were homogenized in a buffer containing 20% glycerol.

†The values of one standard deviation are in parentheses.

the homogenization buffer if the NAD-linked enzyme was to be isolated.[2] The effect of glycerol on the activity of this enzyme is shown in Table I. The 15-ketoprostaglandin Δ^{13} reductase is stable in the absence of glycerol, but 20% glycerol was also present in the homogenization buffer used in the isolation of this enzyme.[3] The NADP-linked 15-hydroxyprostaglandin dehydrogenase and its inhibitor are stable in the absence of glycerol, so that glycerol was not added to the homogenization buffer used to isolate this enzyme and its inhibitor.[4, 5] After homogenization, the homogenates were centrifuged at 10,000 × g for 45 minutes. The homogenization and centrifugation were performed at 0 to 4° C. Assays of the NAD-linked and NADP-linked 15-hydroxyprostaglandin dehydrogenases,[2, 5] the 15-ketoprostaglandin Δ^{13} reductase,[3] and the inhibitor of the NADP-linked enzyme[5] were performed as described previously with the use of dialyzed or undialyzed centrifuged placental homogenates. One unit of enzyme activity is defined as the amount of enzyme which oxidizes or reduces 1 μmol of cofactor per minute under the condition of the assay.

Results

The first step in the metabolism of prostaglandins involves oxidation of the 15-hydroxyl group or oxidoreduction at the 9-position. Placental tissue contains one NAD-linked[1, 2] and four or five NADP-linked[4, 5]

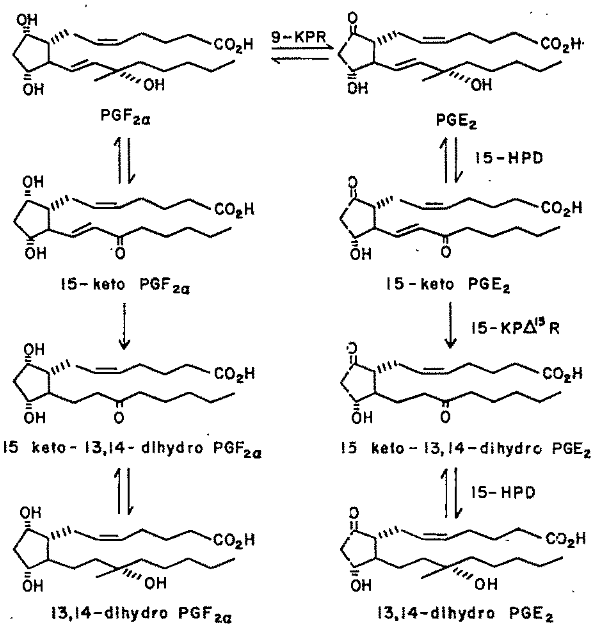

Fig. 1. The early steps in the metabolism of PGE_2 and $PGF_{2\alpha}$ in centrifuged placental homogenates. The enzymes catalyzing the various reactions are abbreviated as follows: 9-ketoprostaglandin reductase, *9-KPR:* NAD-linked 15-hydroxyprostaglandin dehydrogenase, *15-HPD:* 15-ketoprostaglandin Δ^{13} reductase, *15-KPΔ^{13}R.*

15-hydroxyprostaglandin dehydrogenases. The latter enzymes also possess 9-ketoprostaglandin reductase activity.[4, 5]

The most active of these dehydrogenases is the NAD-linked enzyme. The total activity of this enzyme per placenta and the activity per gram of placental tissue are shown in Table I. Four large-scale enzyme preparations, containing 135 placentas and assayed under different conditions, are included in the table. Prostaglandin E_2 is a better substrate for this enzyme than PGE_1.[1] This is not apparent from a comparison of preparations 1 and 2 in Table I and may have been obscured by the wide variation in the enzyme content of individual placentas. The homogenization medium (preparation 3) and the pH of the assay buffer (preparation 4) both have a greater influence on the enzyme activity than does whether the assay is performed with PGE_1 or PGE_2. Although there is considerable variation between individual placentas from twins, cesarean sections, and induced deliveries, there is no significant difference between these placentas and those obtained from spontaneous single deliveries in their content of the NAD-linked enzyme.

Although PGB probably does not occur naturally, it was chosen as a substrate for the NADP-linked 15-hydroxyprostaglandin dehydrogenase* for several reasons: it is a much better substrate for this activity than any other prostaglandin,[5] and it is not a substrate for the NAD-linked enzyme. Thus it is possible to measure the NADP-linked dehydrogenase activity in the presence of the NAD-linked dehydrogenase.† The NADP-linked activity in 66 placentas is shown in Table II. There is no significant difference between placentas

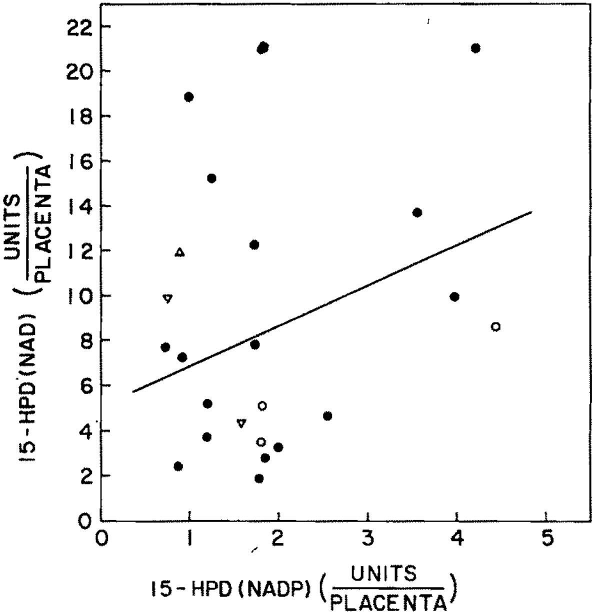

Fig. 2. The relationship between the NAD-linked 15-hydroxyprostaglandin dehydrogenase, *15-HPD(NAD),* and the NADP-linked 15-hydroxyprostaglandin dehydrogenase, *15-HPD(NADP),* activities in centrifuged placental homogenates from spontaneous deliveries (●), induced deliveries(▽), cesarean sections in labor (Δ), and cesarean sections not in labor (o). The spontaneous deliveries (n = 16) were used to derive the regression line ($y = 1.77x + 5.17$) and the correlation coefficient ($r = 0.378$). The value of r was not statistically significant ($P > 0.1$).

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*Disc electrophoresis with histochemical staining has shown that centrifuged placental homogenates contain two major and two or three minor electrophoretic components with NADP-linked 15-hydroxyprostaglandin dehydrogenase activity.[5] It has not been possible to quantitate the activity of these separate components in placental homogenates; consequently, the term NADP-linked 15-hydroxyprostaglandin dehydrogenase will refer to the total NADP-linked activity that is assayed spectrophotometrically.

†The NAD-linked, unlike the NADP-linked, dehydrogenase does not have a unique substrate. Since both enzymes may use NAD as a cofactor, it could be difficult to assay the NAD-linked enzyme in the presence of the NADP-linked enzyme. However, that is not the case in centrifuged placental homogenates, because the activity of the NAD-linked enzyme is more than 7,000 times that of the NADP-linked enzyme when PGE_2 and NAD are used to assay both.

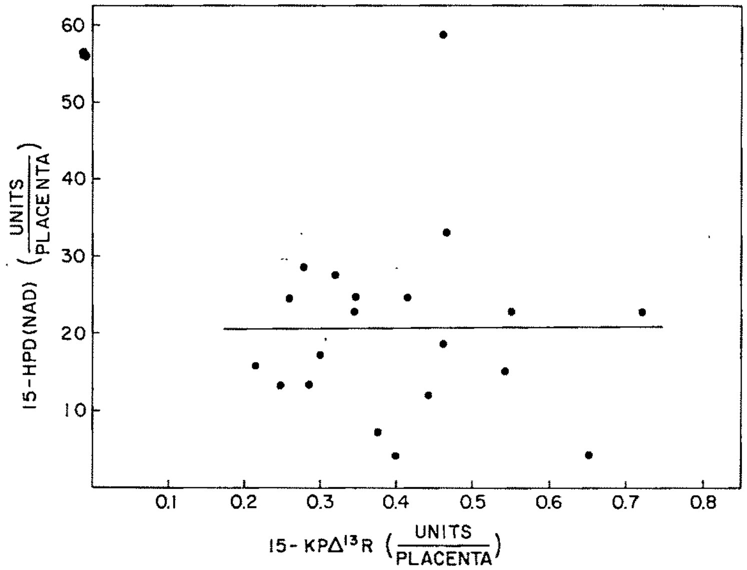

Fig. 3. The relationship between the NAD-linked 15-hydroxyprostaglandin dehydrogenase, *15-HPD(NAD)*, and the 15-ketoprostaglandin Δ^{13} reductase, *15-KPΔ^{13}R*, activities in centrifuged placental homogenates from 20 spontaneous deliveries. These activities were used to derive the regression line ($y = 0.28x + 20.5$) and the correlation coefficient ($r = 0.003$). The value of r was not statistically significant ($P > 0.1$).

Table II. NADP-linked 15-hydroxyprostaglandin dehydrogenase activity in centrifuged placental homogenates*

Assay conditions	*Type of delivery and number of placentas*	*Units/placenta* ($\times 10^3$)	*Units/gm tissue* ($\times 10^3$)
Phosphate buffer pH 7.0 PGB$_1$	Single, spontaneous, normal (54)	2,270 (±1,110)	6.72 (±3.04)
	Single, spontaneous, diabetes (1)	2,950	7.47
	Cesarean section, in labor (3)	876	2.47
		2,710	6.37
		3,200	6.41
	Cesarean section, not in labor, normal (5)	725	1.93
		1,790	5.98
		1,800	4.94
		4,150	9.55
		4,460	11.0
	Cesarean section, not in labor, diabetes (1)	2,130	4.83
	Induced (2)	725	2.73
		1,570	5.23

*The data in this table were obtained from enzyme preparations done in collaboration with Dr. J. Korff and Ms. B. Lin.

from spontaneous deliveries and those from cesarean sections in their content of this enzyme (Table II); and there is no correlation between the activities of the NAD-linked and NADP-linked enzymes in a given placenta (Fig. 2).

The second step in prostaglandin metabolism is the reduction of the Δ^{13} double bond. In centrifuged placental homogenates this reduction is catalyzed by an NADH-linked 15-ketoprostaglandin Δ^{13} reductase[3]; 15-hydroxyprostaglandins are not substrates for this enzyme.[3] The activity of the 15-ketoprostaglandin Δ^{13} reductase in 20 placentas is shown in Table III. There is no correlation between the activity of this enzyme and that of the NAD-linked 15-hydroxyprostaglandin dehydrogenase in a given placenta (Fig. 3). The average activity of the reductase is about one fiftieth of that of the NAD-linked dehydrogenase.

Although the reduction of 15-keto-13,14-dihydroprostaglandins to 13,14-dihydroprostaglandins may be viewed as analogous to a reversal of the oxidation of prostaglandins to 15-ketoprostaglandins, several studies[6, 7] have suggested that the reduction of 15-keto-

Table III. 15-Ketoprostaglandin Δ^{13} reductase activity in centrifuged placental homogenates*

Assay conditions	*Type of delivery and number of placentas*	*Units/placenta* $(\times 10^3)$	*Units/gm tissue* $(\times 10^3)$
1. Phosphate buffer pH 7.0 15-keto PGE_2	Single, spontaneous (20)	406 (±136)	1.20 (±0.36)

*The data for this table were obtained from an enzyme preparation done in collaboration with Dr. C. Westbrook.

Table IV. Substrate specificity of the NAD-linked 15-hydroxyprostaglandin dehydrogenase*

Substrate	*Apparent Km* (μM)	*Relative* V_{max} (%)
PGE_1	2.55	100
13,14-dihydro PGE_1	16.9	49
PGE_2	2.53	103
13,14-dihydro PGE_2	2.80	77
$PGF_{2\alpha}$	13.3	76
15-keto PGE_1	11.8	123
15-keto PGE_2	12.5	82
15-keto-13,14-dihydro PGE_2	8.7	91

*All assays were performed at pH 7.0 in a phosphate buffer[1] with 1.36 μmoles of NAD and variable amounts of the 15-hydroxyprostaglandins or 0.68 μmoles of NADH and variable amounts of the 15-ketoprostaglandins.

13,14-dihydroprostaglandins is catalyzed by 15-ketoprostaglandin reductases, whereas the oxidation of prostaglandins is catalyzed by 15-hydroxyprostaglandin dehydrogenases. The rates of oxidation of prostaglandins and 13,14-dihydroprostaglandins and of reduction of 15-ketoprostaglandins and 15-keto-13,14-dihydroprostaglandin E_2 by a homogeneous preparation[2] of the NAD-linked 15-hydroxyprostaglandin dehydrogenase are shown in Table IV. As may be seen, the 13,14-dihydroprostaglandins and 15-keto-13,14-dihydroprostaglandin E_2 are excellent substrates for this enzyme. The relative rates of these reactions are the same whether centrifuged placental homogenates (not shown) or homogeneous NAD-linked 15-hydroxyprostaglandin dehydrogenase is used as the source of the enzyme, thus suggesting that most, if not all, of the reduction of 15-keto-13,14-dihydroprostaglandins which occurs in centrifuged placental homogenates is catalyzed by the NAD-linked 15-hydroxyprostaglandin dehydrogenase.

A naturally occurring inhibitor of the NADP-linked 15-hydroxyprostaglandin dehydrogenase has been identified in boiled placental homogenates.[5] The relationship between the amount of inhibitor in these boiled homogenates and the NADP-linked activity in unboiled, dialyzed homogenates from the same placentas is shown in Fig. 4.

Comment

All of the enzymes described in this study are pyridine nucleotide linked. When an assay of one of these is performed, it is desirable to add prostaglandin and pyridine nucleotide to the reaction mixture in sufficient concentrations to saturate the enzyme. Although the concentration of the reactants and, consequently, the enzyme activity are higher than those that are observed physiologically, these conditions have been chosen because they will yield zero-order enzyme kinetics. Under such conditions, the initial reaction velocity will reflect the amount of enzyme present.

The rate of oxidation of prostaglandins to 15-ketoprostaglandins is rapid at saturating concentrations of prostaglandin and pyridine nucleotide, and the data in Table I demonstrate that the capacity of the placenta to oxidize prostaglandins at the 15-position greatly exceeds the daily rate of prostaglandin synthesis.* Although placental homogenates contain two enzymes which catalyze the 15-oxidation of prostaglandins, there is no apparent relationship between the activities of these enzymes (Fig. 2), and a comparison of the rate of PGE_2 oxidation by each suggests that the NADP-linked 15-hydroxyprostaglandin dehydrogenase does not play a significant role in the oxidation of PGE_2 in the placenta.† Since the NADP-linked enzyme catalyzes the conversion of PGE_2 to $PGF_{2\alpha}$ at six times the rate of oxidation of PGE_2 to 15-keto PGE_2,[5] it may function physiologically in the placenta as a 9-ketoprostaglandin reductase rather than as a 15-hydroxyprostaglandin dehydrogenase.

A comparison of the enzyme activities given in Tables I, III, and IV suggests that the rate-limiting step in placental prostaglandin metabolism is catalyzed by the 15-ketoprostaglandin Δ^{13} reductase. The facts that the reaction catalyzed by this enzyme is irreversible[3] and that the enzyme is inhibited by NADP and NADPH[3] are consistent with the hypothesis that the 15-ketoprostaglandin Δ^{13} reductase, rather than the NAD-linked 15-hydroxyprostaglandin dehydrogenase, regulates the rate of the early steps of prostaglandin metabolism in the placenta. If that were the case, 15-ketoprostaglandins would be the major prostaglandin metabolites of the placenta. Most studies in which plasma prostaglandin metabolites have been measure during pregnancy have determined only 15-keto-13,14-dihydroprostaglandin levels.[10-12] In one study, however, plasma levels of 15-keto PGE_2 were measured prior to and during the induction of labor with PGE_2.[13] Prior to the administration of PGE_2, 15-keto-PGE_2 was "undetectable" (<6 pg/ml). This is significantly lower than the levels of 15-keto-13,14-dihydro PGE_2 in pregnancy.[11] If subsequent studies confirm this observation, it will remain to be determined whether the placental contribution to the pool of circulating prostaglandin metabolites is small or whether the 15-ketoprostaglandins which have originated in the placenta have been rapidly converted to 15-keto-13,14-dihydroprostaglandins by tissues, such as the lung,[14] in which the 15-ketoprostaglandin Δ^{13} reductase is not rate limiting.

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*The data of Hamberg[8] indicate that the average urinary excretion of the major metabolite of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ increases fourfold to fivefold during pregnancy. If one assumes that this reflects only a change in prostaglandin synthesis, and that PGE synthesis changes similarly, then, from the data of Samuelsson,[9] one can estimate that 90 to 190 μg of $PGE_1 + PGE_2$ and 180 to 305 μg of $PGF_{1\alpha} + PGF_{2\alpha}$ are synthesized daily at term in pregnancy. The data in Table I, preparation 1, indicate that a normal term placenta contains enough NAD-linked enzyme to oxidize more than 10.8 gm of PGE_1 or 8.4 gm of $PGF_{2\alpha}$ per day under optimal conditions.[1]

†See daggered footnote on page 536.

Studies of the 9-ketoprostaglandin reductase activity in rabbit kidney suggest that this enzyme may play a role in regulating the renin-angiotensin system.[15] Other studies suggest that abnormalities in the levels of 15-hydroxyprostaglandin dehydrogenase may be involved in the pathogenesis of spontaneous hypertension in rats.[16] The observation that boiled placental homogenates contain a specific inhibitor of the 9-ketoprostaglandin reductase and the NADP-linked 15-hydroxyprostaglandin dehydrogenase activities[4, 5] led to speculation that this inhibitor might regulate the activity of these enzymes and be involved with the renin-angiotensin system or the control of blood pressure during pregnancy.[4] Although no significant relationship was found between the amount of inhibitor and the NADP-linked 15-hydroxyprostaglandin dehydrogenase activity in 23 placentas (Fig. 4), it should be noticed that these placentas were all from nonhypertensive individuals and that no attempt had been made to control the intake of sodium. Thus additional, carefully controlled studies will be necessary before one may draw conclusions concerning whether the placental inhibitor of the 9-ketoprostaglandin reductase and the NADP-linked 15-hydroxyprostaglandin dehydrogenase has a significant physiologic role.

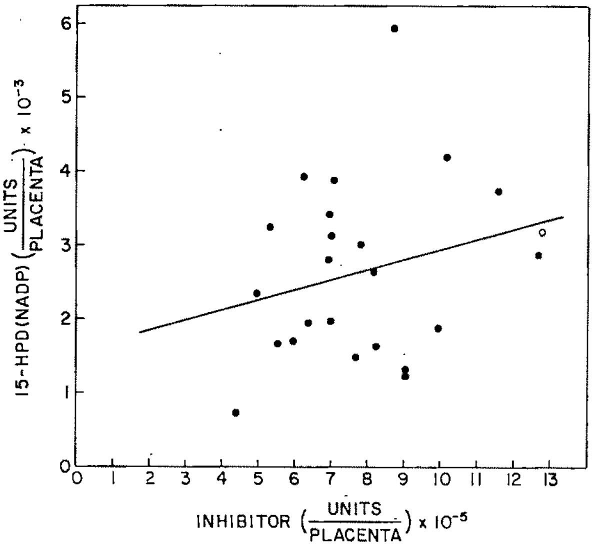

Fig. 4. The relationship between the NADP-linked 15-hydroxyprostaglandin dehydrogenase, *15-HPD(NADP)*, activity in dialyzed centrifuged placental hemogenates and the amount of inhibitor of this enzyme in undialyzed centrifuged placental homogenates from spontaneous deliveries (●) and a cesarean section (○). The spontaneous deliveries ($n = 23$) were used to derive the regression line ($y = 0.137x + 1.6$) and the correlation coefficient ($r = 0.236$). The value of r was not statistically significant ($P > 0.1$).

I wish to thank Dr. J. E. Pike for providing the prostaglandins used in this study, and Drs. D. Shanklin and P. Graff for their assistance in the collection of placentas.

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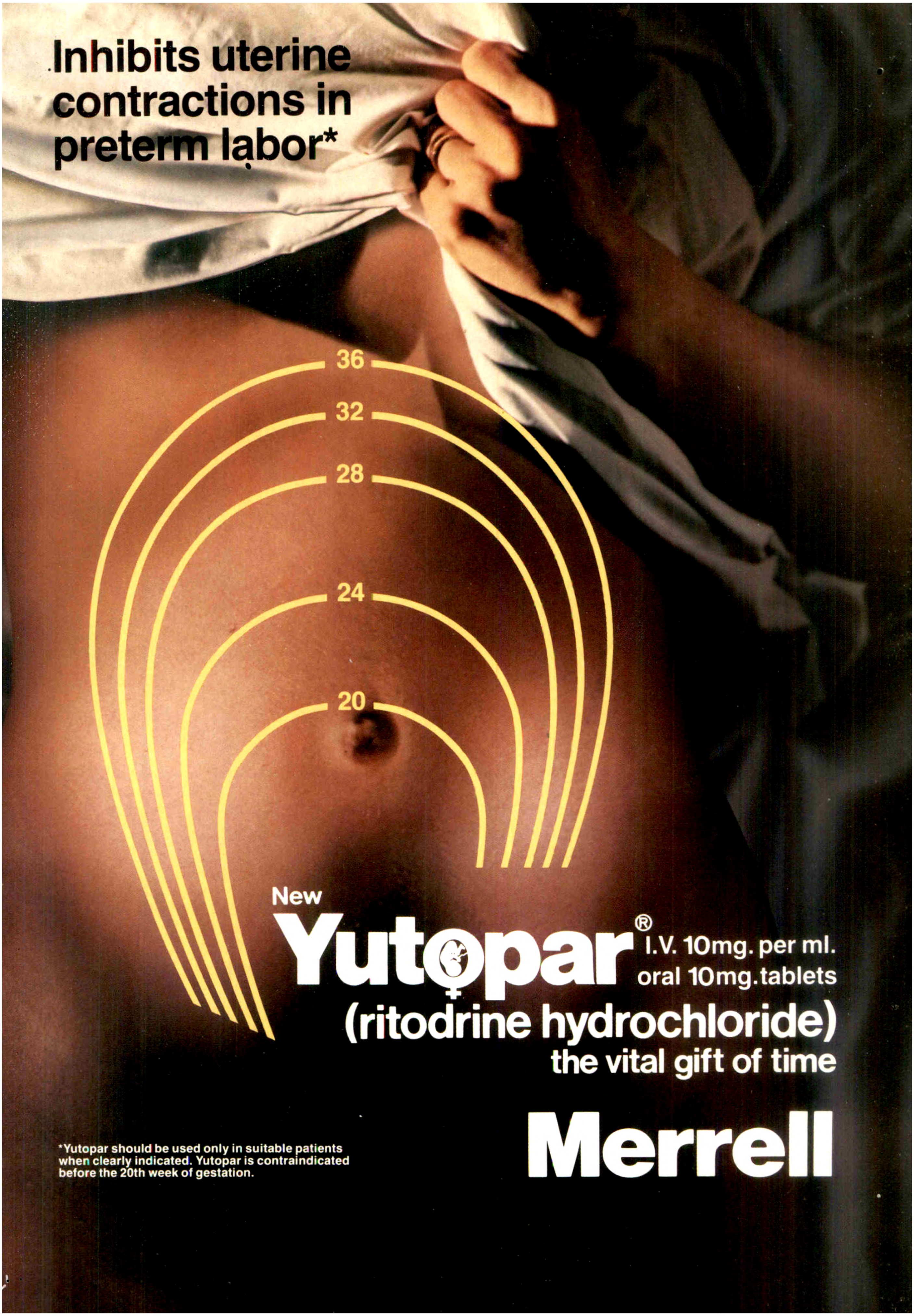

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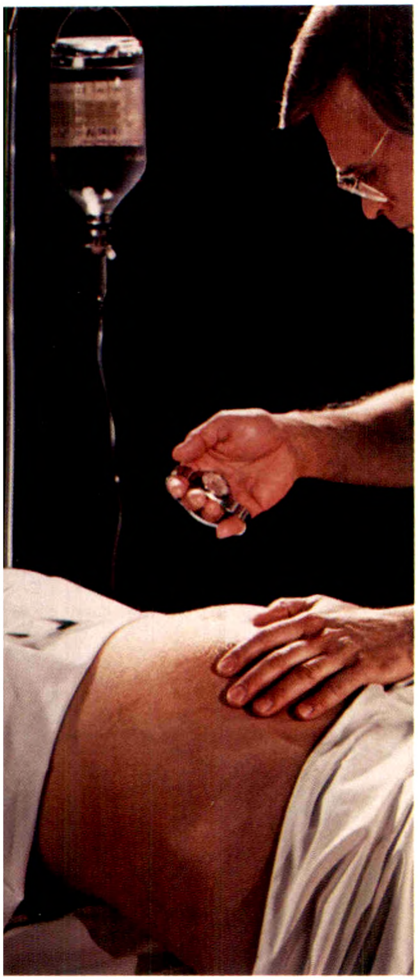


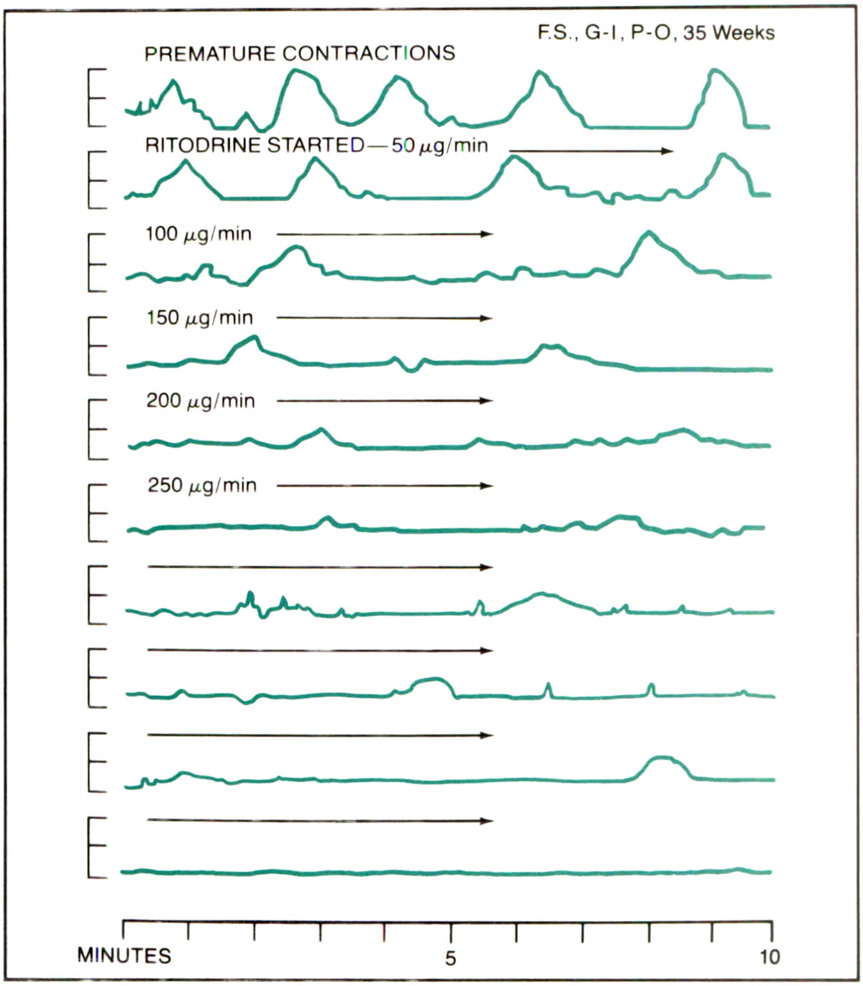


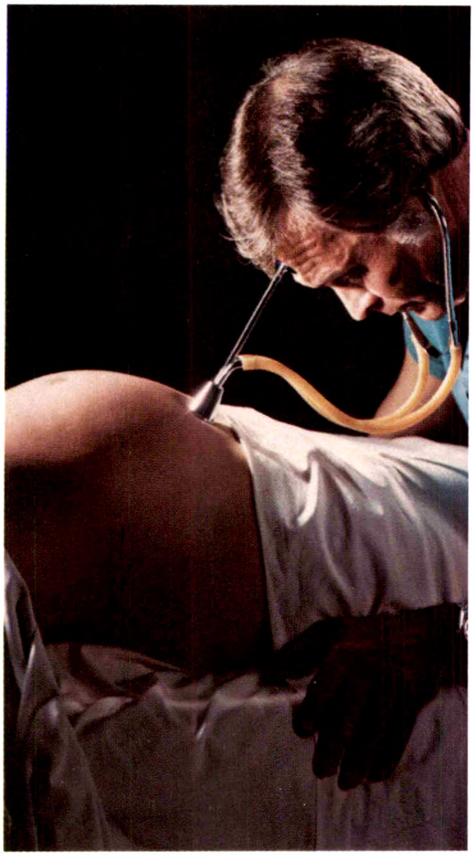
Figure 2. Tracing of the intravenous Yutopar infusion effect on uterine activity from a single patient in preterm labor at 35 weeks. The tracing records, in 10 minute segments monitored by external tocography, uterine activity immediately before and during a 90 minute infusion with the Yutopar dose increasing from 50 to 250 μg. per minute.

From Lauersen *et al.*[1]

Yutopar
the vital gift of time

• Cardiovascular effects:

The effects on the maternal cardiovascular system are dose-related.[3] Marked hypotension is not produced.[3] Cardiac output is increased.[3] Ritodrine passes the placental barrier in sheep[4] and is thought to do so in humans as well. The effects on the human fetal heart rate are modest.[5,6] Because cardiovascular responses are common and most pronounced during intravenous administration of Yutopar, cardiovascular effects, including maternal pulse rate and blood pressure and fetal heart rate, should be closely monitored.



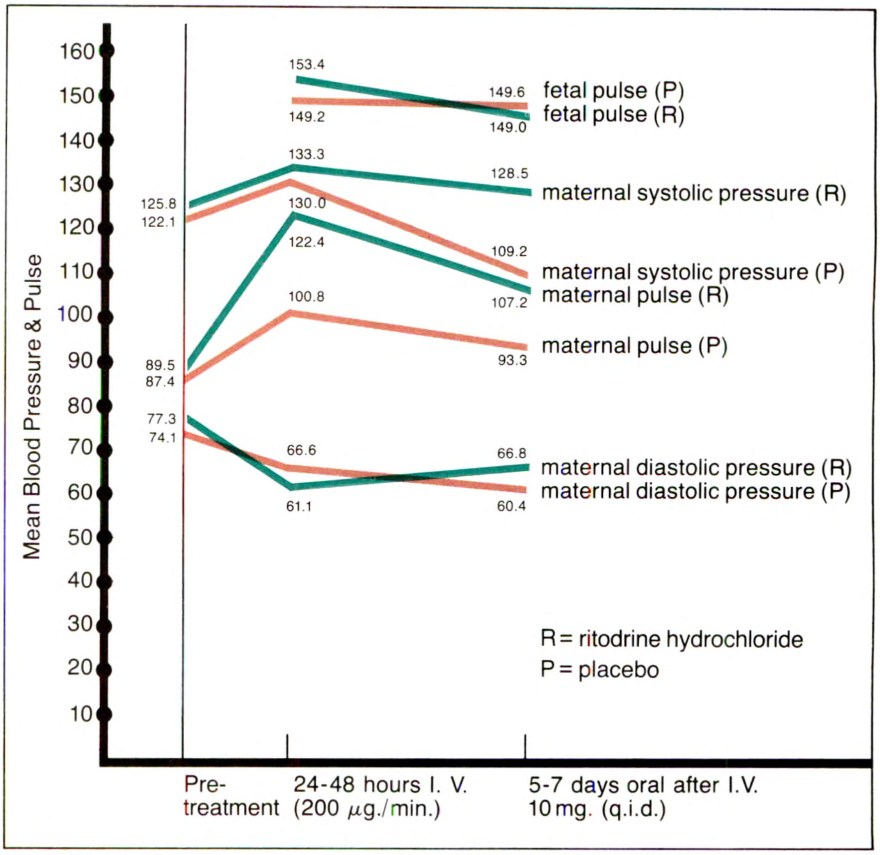

Mean Blood Pressure & Pulse
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
Pre-treatment
24-48 hours I. V. (200 μg./min.)
5-7 days oral after I.V. 10 mg. (q.i.d.)
153.4
149.6
fetal pulse (P)
149.2
149.0
fetal pulse (R)
133.3
128.5
maternal systolic pressure (R)
125.8
122.1
130.0
122.4
109.2
maternal systolic pressure (P)
107.2
maternal pulse (R)
100.8
89.5
87.4
93.3
maternal pulse (P)
77.3
74.1
66.6
66.8
maternal diastolic pressure (R)
61.1
60.4
maternal diastolic pressure (P)
R = ritodrine hydrochloride
P = placebo

Figure 3. The mean maternal pulse pressure and heart rate are shown to increase during intravenous ritodrine treatment and there is a slight rise in the mean systolic blood pressure.[7] These effects are less marked in the control group and show a tendency to subside during oral therapy.

Based on data from Wesselius-DeCasparis *et al.*[7]

• Metabolic effects:

As a consequence of its beta-mimetic mechanism of action, ritodrine intravenously has transient metabolic effects. These may include elevations of blood glucose,[8,9] insulin,[8,9] triglycerides,[10] and free fatty acids.[10] Decreased serum iron and serum potassium have also been found.[10] No effects on other electrolytes have been reported.

References: 1. Barden, T.P., Peter, J.B., and Merkatz, I.R.: Ritodrine Hydrochloride: A Betamimetic Agent for Use in Preterm Labor, I. Pharmacology, Clinical History, Administration, Side Effects, and Safety. Obstet. & Gynecol. 56:1-6, 1980. Merkatz, I.R., Peter, J.B., and Barden, T.P.: Ritodrine Hydrochloride: A Betamimetic Agent for Use in Preterm Labor, II. Evidence of Efficacy. Obstet. & Gynecol. 56:7-12, 1980. 2. Tepperman, H.M., Beydoun, S.N., and Abdul-Karim, R.W.: Drugs affecting myometrial contractility in pregnancy. Clin. Obstet. Gynec. 20:423-445, 1977. 3. Bieniarz, J., Ivankovich, A. and Scommegna, A.: Cardiac output during ritodrine treatment in premature labor. Amer. J. Obstet. Gynec. 118:910-920, 1974. 4. Kleinhout, J., Stolte, L.A.M., and Veth, A.F.L.: Does ritodrine cross the placenta? Nederl. T. Geneesk. 118:1248, 1974. 5. Bieniarz, J.: Cardiovascular effects of beta-adrenergic agonists. In, Pre-Term Labour, Proceedings of the Fifth Study Group of the Royal College of Obstetricians and Gynaecologists 1977, A. Anderson, R. Beard, J.M. Brudenell, P. Dunn, Ed., London. The Royal College of Obstetricians and Gynaecologists 1977, p.188-202. 6. Lipshitz, J. and Baillie, P.: Uterine and cardiovascular effects of $beta_2$-selective sympathomimetic drugs administered as an intravenous infusion. S. Afr. Med. J. 50:1973-1977, 1976. 7. Wesselius-DeCasparis, A., Thiery, M., Yo Le Sian, A., Baumgarten, K., Brosens, I., Gamisans, O., Stolk, J.G., and Vivier, W.: Results of double-blind, multicentre study with ritodrine in premature labour. Brit. Med. J. 3:144-147, 1971. 8. Urban, G., Baumgarten, K., Baumung, H., Beck, A., and Gruber, W.: The influence of ritodrine on glucose and insulin blood levels after oral glucose loading in the last three months of normal pregnancy. Europ. J. Obstet. Gynec. Reprod. Biol. 3:77-79, 1973. 9. Spellacy, W.N., Cruz, A.C., Buhi, W.C., and Birk, S.A.: The acute effects of ritodrine infusion on maternal metabolism: measurements of levels of glucose, insulin, glucagon, triglycerides, cholesterol, placental lactogen, and chorionic gonadotropin. Amer. J. Obstet. Gynec. 131:637-642, 1978. 10. Kauppila, A., Tuimala, R., Ylikorkala, O., Happalahti, J., Karppanen, H., and Viinikka, L.: Effects of ritodrine and isoxsuprine with and without dexamethasone during late pregnancy. Obstet. Gynecol. 51:288-292, 1978.

36
32
28
24
20
Yutopar
the vital gift of time

(Please read the Product Information on the last page before prescribing Yutopar.)

Yutopar®

(ritodrine hydrochloride)

Dosage and Administration Chart

Frequent monitoring of maternal uterine contractions, heart rate, and blood pressure, and of fetal heart rate is required. The dose of Yutopar should be individually titrated according to response.

INTRAVENOUS

Intravenous administration of Yutopar should be supervised by persons having knowledge of the pharmacology of the drug and qualified to identify and manage complications of drug administration and pregnancy.

The patient receiving intravenous therapy should be kept in the left lateral recumbent position.

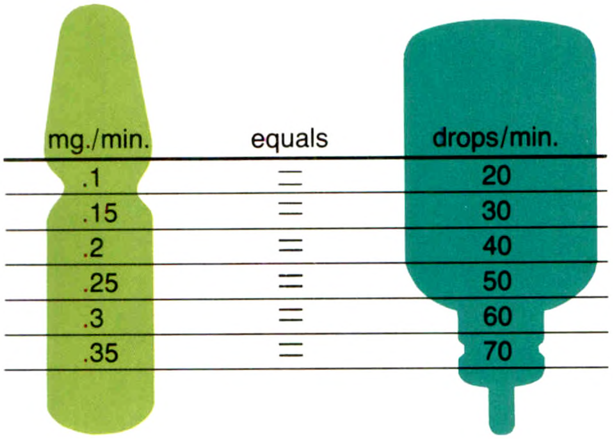
Solution: dilute 150 mg. ritodrine hydrochloride (3 ampuls) in 500 ml. solution, which yields a final concentration of 0.3 mg./ml. (Do not use parenteral Yutopar if the solution is discolored or contains any precipitate or particulate matter.) The following solutions may be used for dilution:

0.9% w/v sodium chloride
5% w/v dextrose
10% w/v Dextran 40 in
0.9% w/v sodium chloride

10% w/v invert sugar
Cpd sodium chloride (Ringer's)
Hartmann's

Method of Administration: a controlled infusion device is recommended to adjust the rate of flow in drops per minute. An i.v. microdrip chamber 60 drops/ml. can provide a convenient range of infusion rates within the recommended dose for Yutopar.

Dosage: the usual initial dose is 0.1 mg./minute, to be increased according to results by 0.05 mg./minute every 10 minutes until the desired result is obtained. The effective dosage usually lies between 0.15 and 0.35 mg./minute. The infusion should generally be continued for at least 12 hours after uterine contractions cease.



mg./min.	equals	drops/min.
.1	=	20
.15	=	30
.2	=	40
.25	=	50
.3	=	60
.35	=	70

Dosage equivalents

(at dilution of 3 ampuls/500 ml. fluid)
if microdrip chamber (60 drops/ml.) is used

Note: At the recommended dilution and rate of administration, the maximum volume of fluid which might be administered over 12 hours at the highest dose (0.35 mg./min.) will be approximately 840 ml. The total amount of i.v. fluids administered and the rate of administration should be monitored to avoid circulatory fluid overload.

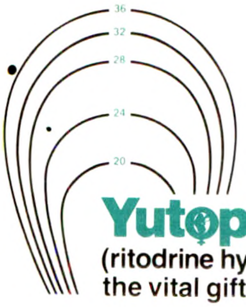
Recurrence: may be treated with repeated infusions, as above

ORAL

Maintenance: 1 tablet (10 mg.) approximately 30 minutes before termination of i.v. therapy. For the first 24 hours the usual dosage is 1 tablet every 2 hours. Thereafter, the usual dosage is 1 or 2 tablets (10 to 20 mg.) every 4 to 6 hours, depending on uterine activity and side effects. Total daily dose should not exceed 120 mg.

(Please read the Product Information on the last page before administering Yutopar.)

Merrell



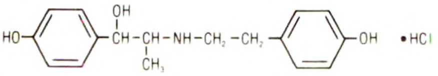
Yutopar®
(ritodrine hydrochloride)
the vital gift of time

CAUTION: Federal law prohibits dispensing without prescription

DESCRIPTION

Yutopar, which contains the betamimetic (beta sympathomimetic amine) ritodrine hydrochloride, is available in two dosage forms. Yutopar for parenteral (intravenous) use is a clear, colorless, sterile, aqueous solution; each milliliter contains 10 mg. of ritodrine hydrochloride, 0.44% (w/v) of acetic acid, 0.24% (w/v) of sodium hydroxide, 0.1% (w/v) of sodium metabisulfite, and 0.29% (w/v) of sodium chloride in water for injection USP. Hydrochloric acid or additional sodium hydroxide is used to adjust pH. Each Yutopar tablet contains 10 mg. of ritodrine hydrochloride.

Ritodrine hydrochloride is a white, odorless crystalline powder, freely soluble in water, with a melting point between 193° and 201°C. The chemical name of ritodrine hydrochloride is erythro-p-hydroxy-α-[1-[(p-hydroxyphenethyl) amino]ethyl] benzyl alcohol hydrochloride and has the chemical structure:



CLINICAL PHARMACOLOGY

Yutopar (ritodrine hydrochloride) is a beta-receptor agonist, which has been shown by *in vitro* and *in vivo* pharmacologic studies in animals to exert a preferential effect on the β_2 adrenergic receptors such as those in the uterine smooth muscle. Stimulation of the β_2 receptors inhibits contractility of the uterine smooth muscle.

In humans, intravenous infusions of 0.05 to 0.30 mg./min. or single oral doses of 10 to 20 mg. decreased the intensity and frequency of uterine contractions. These effects were antagonized by beta-blocking compounds. Intravenous administration induced an immediate dose-related elevation of heart rate with maximum mean increases between 19 and 40 beats per minute. Widening of the pulse pressure was also observed; the average increase in systolic blood pressure was 4.0 mm. Hg, and the average decrease in diastolic pressure was 12.3 mm. Hg. With oral intake, the increase in heart rate was mild and delayed.

During intravenous infusion in humans, transient elevations of blood glucose, insulin, and free fatty acids have been observed. Decreased serum potassium has also been found, but effects on other electrolytes have not been reported.

Serum kinetics in humans (non-pregnant females) of an intravenous infusion of 60 minutes duration were determined by measuring serum ritodrine levels by a radioimmunoassay technique. Three half-lives were calculated: the first of 6 to 9 minutes dominated the ascending phase of the serum drug-level curve; the second phase of 1.7 to 2.6 hours dominated the descending curve; and finally, a third phase, with a half-life of more than 10 hours, was discernible. In a study of serum kinetics after oral ingestion (male subjects), the decline of serum drug levels could be described in terms of a two-phase decay with an initial half-life of 1.3 hours and a final half-life of 12 hours. With either route of administration, 90% of the excretion was completed within 24 hours after the dose.

Comparison of ritodrine serum levels after intravenous administration with those after oral dosage indicates the oral bioavailability is about 30%. Intravenous infusion at a rate of 0.15 mg./min. for 1 hour yielded maximum serum levels ranging between 32 and 52 ng./ml. in a group of 6 non-pregnant female volunteers; maximum serum levels following single and repeated (4 x 10 mg./24 hr.) 10 mg. oral doses ranged between 5 and 15 ng./ml. and were obtained within 30 to 60 minutes after ingestion.

Placental transfer was confirmed by measurement of drug concentrations in cord blood showing that ritodrine and its conjugates reach the fetal circulation.

INDICATIONS AND USAGE

Yutopar is indicated for the management of preterm labor in suitable patients.

Administered intravenously, the drug will decrease uterine activity and thus prolong gestation in the majority of such patients. After intravenous Yutopar has arrested the acute episode, oral administration may help to avert relapse. Additional acute episodes may be treated by repeating the intravenous infusion. The incidence of neonatal mortality and respiratory distress syndrome increases when the normal gestation period is shortened.

Since successful inhibition of labor is more likely with early treatment, therapy with Yutopar should be instituted as soon as the diagnosis of preterm labor is established and contraindications ruled out in pregnancies of 20 or more weeks' gestation. The efficacy and safety of Yutopar in advanced labor, that is, when cervical dilatation is more than 4 cm. or effacement is more than 80%, have not been established.

CONTRAINDICATIONS

Yutopar is contraindicated before the 20th week of pregnancy.

Yutopar is also contraindicated in those conditions of the mother or fetus in which continuation of pregnancy is hazardous; specific contraindications include:

1. Antepartum hemorrhage which demands immediate delivery
2. Eclampsia and severe preeclampsia
3. Intrauterine fetal death
4. Chorioamnionitis
5. Maternal cardiac disease
6. Pulmonary hypertension
7. Maternal hyperthyroidism
8. Uncontrolled maternal diabetes mellitus (See Precautions.)
9. Pre-existing maternal medical conditions that would be seriously affected by the known pharmacologic properties of a betamimetic drug; such as: hypovolemia, cardiac arrhythmias associated with tachycardia or digitalis intoxication, uncontrolled hypertension, pheochromocytoma, bronchial asthma already treated by betamimetics and/or steroids
10. Known hypersensitivity to any component of the product

WARNINGS

Maternal pulmonary edema has been reported rarely in patients treated concomitantly with Yutopar and corticosteroids and one maternal death has been reported in such a patient. Patients so treated must be closely monitored in hospital. If pulmonary edema develops, the drugs should be discontinued and the edema managed by conventional means.

Intravenous administration of Yutopar should be supervised by persons having knowledge of the pharmacology of the drug and who are qualified to identify and manage complications of drug administration and pregnancy. Because cardiovascular responses are common and more pronounced during intravenous administration of Yutopar, cardiovascular effects, including maternal pulse rate and blood pressure and fetal heart rate, should be closely monitored. Care should be exercised for maternal signs and symptoms of pulmonary edema. Occult cardiac disease may be unmasked with the use of Yutopar.

The drug should not be administered to patients with mild to moderate preeclampsia, hypertension, or diabetes unless the attending physician considers that the benefits clearly outweigh the risks.

PRECAUTIONS

When Yutopar is used for the management of preterm labor in a patient with premature rupture of the membranes, the benefits of delaying delivery should be balanced against the potential risks of development of chorioamnionitis.

Among low birth weight infants, approximately 9% may be growth retarded for gestational age. Therefore, Intra-Uterine Growth Retardation (IUGR) should be considered in the differential diagnosis of preterm labor; this is especially important when the gestational age is in doubt. The decision to continue or reinitiate the administration of Yutopar will depend on an assessment of fetal maturity. In addition to clinical parameters, other studies, such as sonography or amniocentesis, may be helpful in establishing the state of fetal maturity if it is in doubt.

Laboratory Tests

Because intravenous administration of Yutopar has been shown to elevate plasma insulin and glucose and to decrease plasma potassium concentrations, monitoring of glucose and electrolyte levels is recommended during protracted infusions. Special attention should be paid to biochemical variables when treating diabetic patients or those receiving potassium-depleting diuretics.

Drug Interactions

Corticosteroids used concomitantly may lead to pulmonary edema. (See Warnings.)

The effects of other sympathomimetic amines may be potentiated when concurrently administered and these effects may be additive. A sufficient time interval should elapse prior to administration of another sympathomimetic drug.

Beta-adrenergic blocking drugs inhibit the action of Yutopar; coadministration of these drugs should, therefore, be avoided.

With anesthetics used in surgery, the possibility that hypotensive effects may be potentiated should be considered.

Carcinogenesis

In rats given oral doses of 1, 10, and 150 mg./kg./day of ritodrine hydrochloride for 82 weeks, no evidence of tumorigenicity was noted.

Pregnancy

Teratogenic Effects

(Pregnancy Category B)

Reproduction studies were performed in rats and rabbits. The doses employed intravenously were 1/9 (1 mg./kg.), 1/3 (3 mg./kg.), and 1 (9 mg./kg.) times the maximum human daily intravenous dose (but given to the animals as a bolus rather than by infusion). The oral doses, 10 and 100 mg./kg., represented 5 and 50 times the maximum human daily oral maintenance dose. The results of these studies have revealed no evidence of impaired fertility or harm to the fetus due to ritodrine hydrochloride.

No adverse fetal effects were encountered when single intravenous doses of 1, 3, and 9 mg./kg./day or oral doses of 10 and 100 mg./kg./day were given to rats and rabbits on Days 6 through 15 and 6 through 18 of gestation, respectively. Intravenous doses of 1 and 8 mg./kg./day or oral doses of 10 and 100 mg./kg./day administered to the mother from Day 15 of pregnancy to Day 21 postpartum did not affect perinatal or postnatal development in rats. A slight increase in fetal weight in the rat was observed. Oral administration to both sexes did not impair fertility or reproductive performance. Lethal doses to pregnant rats did not cause immediate fetal demise. There are no adequate and well-controlled studies of Yutopar effects in pregnant women before 20 weeks' gestation; therefore, this drug should not be used before the 20th week of pregnancy. Studies of Yutopar administered to pregnant women from the 20th week of gestation have not shown increased risk of fetal abnormalities. Follow-up of selected variables in a small number of children for up to 2 years has not revealed harmful effects on growth, developmental or functional maturation. Nonetheless, although clinical studies did not demonstrate a risk of permanent adverse fetal effects from Yutopar, the possibility cannot be excluded; therefore, Yutopar should be used only when clearly indicated.

Some studies indicate that infants born before 36 weeks' gestation make up less than 10% of all births but account for as many as 75% of perinatal deaths and one-half of all neurologically handicapped infants. There are data available indicating that infants born at any time prior to full term may manifest a higher incidence of neurologic or other handicaps than occurs in the total population of infants born at or after full term. In delaying or preventing preterm labor, the use of Yutopar should result in an overall increase in neonatal survival. Handicapped infants who might not have otherwise survived may survive.

ADVERSE REACTIONS

The unwanted effects of Yutopar are related to its betamimetic activity and usually are controlled by suitable dosage adjustment.

Effects Associated with Intravenous Administration

Usual effects (80-100% of patients)

Intravenous infusion of Yutopar leads almost invariably to dose-related alterations in maternal and fetal heart rates and in maternal blood pressure. During clinical studies in which the maximum infusion rate was limited to 0.35 mg./min. (one patient received 0.40 mg./min.), the maximum maternal and fetal heart rates averaged, respectively, 130 (range 60 to 180) and 164 (range 130 to 200) beats per minute. The maximum maternal systolic blood pressures averaged 128 mm. Hg (range 96 to 162 mm. Hg), an average increase of 12 mm. Hg from pretreatment levels. The minimum maternal diastolic blood pressures averaged 48 mm. Hg (range 0 to 76 mm. Hg), an average decrease of 23 mm. Hg from pretreatment levels. While the more severe effects were usually managed effectively by dosage adjustments, in less than 1% of patients, persistent maternal tachycardia or decreased diastolic blood pressure required withdrawal of the drug.

Yutopar infusion is associated with transient elevation of blood glucose and insulin, which decreases toward normal values after 48 to 72 hours despite continued infusion. Elevation of free fatty acids and cAMP has been reported. Reduction of potassium levels should be expected; other biochemical effects have not been reported.

Frequent effects (10-50% of patients)

Intravenous Yutopar, in about one-third of the patients, was associated with palpitation. Tremor, nausea, vomiting, headache, or erythema was observed in 10 to 15% of patients.

Occasional effects (5-10% of patients)

Nervousness, jitteriness, restlessness, emotional upset, or anxiety was reported in 5 to 6% of patients and malaise in similar numbers.

Infrequent effects (1-3% of patients)

Cardiac symptoms including chest pain or tightness (rarely associated with abnormalities of ECG) and arrhythmia were reported in 1 to 2% of patients.

Other infrequently reported maternal effects included: anaphylactic shock, rash, heart murmur, epigastric distress, ileus, bloating, constipation, diarrhea, dyspnea, hyperventilation, hemolytic icterus, glycosuria, lactic acidosis, sweating, chills, drowsiness, and weakness.

Neonatal Effects

Infrequently reported neonatal symptoms include hypoglycemia and ileus. In addition, hypocalcemia and hypotension have been reported in neonates whose mothers were treated with other betamimetic agents.

Effects Associated with Oral Administration

Frequent effects (<50% of patients)

Oral ritodrine in clinical studies was often associated with small increases in maternal heart rate, but little or no effect upon either maternal systolic or diastolic blood pressure or upon fetal heart rate was found.

Oral ritodrine in 10 to 15% of patients was associated with palpitation or tremor. Nausea and jitteriness were less frequent (5 to 8%), while rash was observed in some patients (3 to 4%), and arrhythmia was infrequent (about 1%).

OVERDOSAGE

The symptoms of overdosage are those of excessive beta-adrenergic stimulation including exaggeration of the known pharmacologic effects, the most prominent being tachycardia (maternal and fetal), palpitation, cardiac arrhythmia, hypotension, dyspnea, nervousness, tremor, nausea, and vomiting. If an excess of ritodrine tablets is ingested, gastric lavage or induction of emesis should be carried out followed by administration of activated charcoal. When symptoms of overdose occur as a result of intravenous administration, ritodrine should be discontinued; an appropriate beta-blocking agent may be used as an antidote. Ritodrine hydrochloride is dialyzable.

Acute intravenous toxicity was studied in rats and rabbits and acute oral toxicity in mice, rats, guinea pigs, and dogs. The LD50 values in the most sensitive of the species used were 64 mg./kg. intravenously in the nonpregnant rabbit and 540 mg./kg. orally in the nonpregnant mouse. The intravenous LD50 value in the pregnant rat was 85 mg./kg. The amount of drug required to produce symptoms of overdose in humans is individually variable. No reports of human mortality due to overdose have been received.

DOSAGE AND ADMINISTRATION

In the management of preterm labor, the initial intravenous treatment should usually be followed by oral administration. The optimum dose of Yutopar is determined by a clinical balance of uterine response and unwanted effects.

Intravenous Therapy

Do not use intravenous Yutopar if the solution is discolored or contains any precipitate or particulate matter.

Yutopar solution should be used promptly after preparation, but in no case after 48 hours of preparation.

Method of Administration: To minimize the risks of hypotension, the patient should be maintained in the left lateral position during infusion and careful attention given to her hydration.

For appropriate control and dose titration, a controlled infusion device is recommended to adjust the rate of flow in drops/minute. An i.v. microdrip chamber (60 drops/ml.) can provide a convenient range of infusion rates within the recommended dose range for Yutopar.

Recommended Dilution: 150 mg. ritodrine hydrochloride (3 ampuls) in 500 ml. fluid yielding a final concentration of 0.3 mg./ml. Ritodrine for intravenous infusion should be diluted with one of the following:

- 0.9% w/v sodium chloride solution
- 5% w/v dextrose solution
- 10% w/v dextran 40 in 0.9% w/v sodium chloride solution
- 10% w/v invert sugar solution
- Compound sodium chloride solution (Ringer's solution)
- Hartmann's solution

Intravenous therapy should be started as soon as possible after diagnosis. The usual initial dose is 0.1 mg./minute (0.33 ml./min., 20 drops/min. using a microdrip chamber at the recommended dilution), to be gradually increased according to the results by 0.05 mg./minute (0.17 ml./min., 10 drops/min. using a microdrip chamber at the recommended dilution) every 10 minutes until the desired result is attained. The effective dosage usually lies between 0.15 and 0.35 mg./minute (0.50 to 1.17 ml./min., 30-70 drops/min. using a microdrip chamber at the recommended dilution). The infusion should generally be continued for at least 12 hours after uterine contractions cease. With the recommended dilution, the maximum volume of fluid that might be administered after 12 hours at the highest dose (0.35 mg./min.) will be approximately 840 ml. The amount of i.v. fluids administered and the rate of administration should be monitored to avoid circulatory fluid overload. Frequent monitoring of maternal uterine contractions, heart rate, and blood pressure, and of fetal heart rate is required, with dosage individually titrated according to response.

Oral Maintenance

One tablet (10 mg.) may be given approximately 30 minutes before the termination of intravenous therapy. The usual dosage schedule for the first 24 hours of oral maintenance is 1 tablet (10 mg.) every two hours. Thereafter the usual dose is 1 or 2 tablets (10 to 20 mg.) every four to six hours, the dose depending on uterine activity and unwanted effects. The total daily dose of oral ritodrine should not exceed 120 mg. The treatment may be continued as long as the physician considers it desirable to prolong pregnancy.

Recurrence of unwanted preterm labor may be treated with repeated infusion of Yutopar.

HOW SUPPLIED

NDC 0068-0599-53:	5 ml. ampuls in boxes of 12. Each ampul contains 50 mg. (10 mg./ml.) of ritodrine hydrochloride.
NDC 0068-0595-60:	10 mg. tablets in bottles of 60. Each round, yellow tablet contains 10 mg. ritodrine hydrochloride and is inscribed YUTOPAR on one side and scored on the other.

Product Information as of July, 1980

Both the tablet and intravenous dosage forms should be stored at room temperature, preferably below 86°F. (30°C.). Protect from excessive heat.

Parenteral (intravenous) dosage form manufactured
by TAYLOR PHARMACAL COMPANY
Decatur, Illinois 62525
Distributed by
MERRELL-NATIONAL LABORATORIES
Division of Richardson-Merrell Inc.
Cincinnati, Ohio 45215, U.S.A.

Yutopar® licensed by
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Amsterdam, Holland

Merrell

0-5690 (Y556B)

AUGUST, 1980

PRINTED IN U.S.A.

GYNECOLOGY

Contraceptive practice and tuboovarian abscess

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Research Triangle Park and Chapel Hill, North Carolina

The hospital and operative records of 318 women with a discharge diagnosis of tuboovarian abscess (TOA) were reviewed to evaluate the possible relationship between contraceptive usage and the occurrence of TOA. A significantly higher ($p < 0.10$) proportion of the intrauterine contraceptive device (IUD) users had acute pelvic inflammatory disease and a significantly lower proportion ($p < 0.10$) compared to users of other contraceptive methods were surgically treated. The proportions of women with unilateral and bilateral TOAs were similar, regardless of the contraceptive method used. This was true whether diagnosis was based on physical examination alone or confirmed at operation. (AM. J. OBSTET. GYNECOL. 138:541, 1980.)

RESEARCH during the past decade has led to a consensus of opinion that there is an association between the use of intrauterine contraceptive devices (IUDs) and pelvic inflammatory disease (PID). Although some investigators have expressed the opinion that PID may be more severe among IUD users than among nonusers, there is little objective evidence to either confirm or refute this point of view. One objective criterion of severe PID is the formation of an abscess. Several investigators have reported a cause-and-effect relationship between IUD use and tuboovarian abscesses (TOAs), while others have not found such a relationship. In addition, some reports have suggested that unilateral TOAs occur more frequently among IUD users than among nonusers, while other studies have shown no relationship between IUD use and either unilateral or bilateral TOA formation. These varying opinions have been reviewed previously in detail.[1]

Table I. Contraceptive methods reported by women with TOA

Contraceptive method	*No.*	*%*
None	159	50.0
Oral contraceptive	45	14.2
IUD	67	21.1
Sterilization	24	7.5
Barrier	13	4.1
Unknown	10	3.1

Since 1972, the occurence of TOA (bilateral or unilateral) among IUD users has been documented in more than 20 case reports and other studies. Only three of these reports[2-4] included the appropriate data to ascertain whether IUD users are indeed at an increased risk of bilateral or unilateral TOAs.

Golde and co-workers[2] reported significantly higher ($p < 0.05$) rates of unilateral TOAs among IUD users (62.5%) compared to nonusers (32.1%). On the other hand, Golditch and Huston[3] reported that 17 (54.8%) of 31 patients admitted with a diagnosis of tuboovarian or pelvic abscess were IUD users. This percentage of

From the International Fertility Research Program, Research Triangle Park, and the Department of Obstetrics and Gynecology, University of North Carolina, Chapel Hill.

Supported in part by the International Fertility Research Program and the Office of Population, United States Agency for International Development (AID/pha-C-1172).

Received for publication May 15, 1980.

Revised July 10, 1980.

Accepted July 14, 1980.

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Table II. Characteristics of women with TOA by contraceptive method

Characteristic	*Contraceptive method* *None* (*N = 159*)	*Oral contraceptives* (*N = 45*)	*IUD* (*N = 67*)	*Sterilization* (*N = 24*)	*Barrier* (*N = 13*)	*Unknown* (*N = 10*)
Mean age (yr)	26.4	24.6	24.5	30.0	25.5	33.7
Nulliparous patients (%)	46.5	26.7	29.9	4.2	46.2	40.0
Patients with prior history of PID (%)	60.4	57.8	23.9	58.3	69.2	60.0
Patients admitted with acute PID (%)	39.6	44.4	79.1	37.5	61.5	20.0
Patients with positive gonorrhea culture (%)	18.2	28.9	19.4	12.5	23.1	20.0
Management of abscess (%):						
Surgical	49.7	48.9	25.4	66.7	23.1	60.0
Antibiotics alone	50.3	51.1	74.6	33.3	76.9	40.0

Table III. Types of IUDs among women with TOA

	No.	%
Dalkon Shield	24	35.8
Lippes Loop	17	25.4
Cu-7	7	10.4
Others	4	6.0
Unknown	15	22.4

IUD users was not significantly different (p > 0.10) from the percentage of IUD users (55.7%) among 235 selected patients seen for gynecologic examination at the same hospital. Scott[4] found that the proportion of women with unilateral or bilateral pelvic abscesses was similar (p > 0.10) for both IUD users and nonusers. Among women hospitalized with a diagnosis of PID, 19 (31.6%) of 60 IUD users compared to 47 (39.5%) of 119 nonusers had abscesses. The abscesses were unilateral among 13.3% of the IUD users compared to 16.8% of the nonusers.

The present investigation was undertaken in an attempt to clarify the relationships between contraceptive usage and the occurrence of TOAs.

Material and methods

From January, 1970, to June, 1979, 346 women who were treated as inpatients at the North Carolina Memorial Hospital were discharged with a diagnosis of TOA. The hospital records of these women were reviewed retrospectively and pertinent information was abstracted to evaluate the association between contraceptive practice and TOA. The "diagnosis" of TOA was made by review of the operative notes and pathology reports for patients surgically treated or by review of the physicians' reports contained in the hospital records for patients who were not surgically treated. Generally, the diagnosis of a TOA was made in the presence of either a unilateral or bilateral, tender pelvic mass and the presence of signs or symptoms of an inflammatory process, e.g., fever, elevated white blood cell count, or elevated erythrocyte sedimentation rate. In some cases, the findings of pelvic ultrasonography or culdocentesis were consistent with the diagnosis of TOA. Twenty-eight women were excluded from analysis because they were either premenarchal or postmenopausal or because they developed TOAs following obstetric delivery or following pelvic surgery. In these 28 cases there could have been no apparent relationship of the pelvic infection to contraceptive use.

Results

The contraceptive method in use at the time of hospital admission as recorded in the hospital records is shown in Table I. Most women (50%) did not report active contraceptive use. IUD users accounted for over one fifth of the population.

Selected characteristics of women using each contraceptive method are shown in Table II. Because of the small numbers of women in the sterilization, barrier, and unknown contraceptive practice groups, comparisons were made only between women using IUDs or oral contraceptives and those using no contraceptive method. Compared to women using oral contraceptives or no contraceptive method, a significantly lower (p < 0.10) proportion of the IUD users had a previous history of PID, and a significantly higher (p < 0.10) proportion of IUD users were admitted with acute PID and were treated with antibiotic therapy alone. Among women who required surgical treatment, total abdominal hysterectomy with bilateral salpingo-oophorectomy was carried out for 92.4%, and unilateral or bilateral salpingo-oophorectomy was performed in 7.6%. Other surgical procedures performed included diagnostic laparoscopy (35 cases), exploratory

Table IV. Characteristics of IUD users with TOAs by type of IUD

Characteristic	*Type of IUD Used*		
	Dalkon Shield (N = 24)	*Lippes Loop (N = 17)*	*Others/unknown (N = 26)*
Mean age (yr)	23.8	23.3	27.1
Nulliparous patients (%)	25.0	29.4	34.6
Patients with prior history of PID (%)	29.2	35.3	15.4
Patients admitted with acute PID (%)	79.2	70.6	80.8
Patients with positive gonorrhea culture (%)	16.7	17.6	23.1
Management of abscess (%):			
Surgical	25.0	23.5	26.9
Antibiotics alone	75.0	76.5	73.1
Median duration of IUD use (months)	36.0	30.0	12.2

Table V. Relative frequency of unilateral and bilateral TOAs by contraceptive method

Diagnosis of TOA	*Contraceptive method*					
	None (%)	*Oral contraceptives (%)*	*IUD (%)*	*Sterilization (%)*	*Barrier (%)*	*Unknown (%)*
Surgical:	N = 100	N = 23	N = 21	N = 19	N = 28	N = 8
Unilateral	22.0	17.4	19.1	15.8	12.5	37.5
Bilateral	78.0	82.6	80.8	84.2	87.5	62.5
Physical examination only:	N = 59	N = 22	N = 46	N = 5	N = 5	N = 2
Unilateral	20.3	9.1	28.3	40.0	40.0	0.0
Bilateral	74.6	77.3	71.7	60.0	40.0	100.0
Unknown	5.1	13.6	0.0	0.0	20.0	0.0

Table VI. Relative frequency of unilateral and bilateral TOAs among IUD users

Diagnosis of TOA	*Type of IUD*		
	Dalkon Shield (%)	*Lippes Loop (%)*	*Others/unknown (%)*
Surgical:	N = 8	N = 5	N = 8
Unilateral	25.0	20.0	12.5
Bilateral	75.0	80.0	87.5
Physical examination only:	N = 17	N = 12	N = 18
Unilateral	37.5	25.0	22.2
Bilateral	62.5	75.0	77.8

laparotomy (3 cases), and colpotomy (1 case). The general approach used for the treatment of patients with a diagnosis of TOA was first to treat with antibiotics. If the response was not adequate, the patient was treated surgically. The types of IUDs used are shown in Table III. Dalkon Shields and Lippes Loops were the most commonly used IUDs in this population of women.

Users of Dalkon Shields and Lippes Loops were similar ($p > 0.10$) with respect to selected characteristics (Table IV). The duration of IUD use ranged from less than 1 month to 10 years for Lippes Loop users and from 5 months to 6 years for Dalkon Shield users. For two women (3.8%), both Lippes Loop users, the TOAs were diagnosed within 2 weeks of the IUD insertion.

For all women, TOAs were bilateral in 76.4%, unilateral in 21.4%, and unknown in 2.2% of the cases. The distribution of cases of bilateral versus unilateral TOA was not significantly different ($p > 0.10$) for different contraceptive methods, regardless of the criteria for diagnosis either by physical examination alone or confirmed at operation (Table V). Similarly, the proportions of women with unilateral or bilateral TOA were similar for users of Lippes Loops, Dalkon Shields, and other or unknown types of IUDs (Table VI).

Cervical cultures for gonorrhea were routinely obtained for all women. Overall, 19.8% of the women had positive cultures. The proportions of women with positive cultures were not significantly different ($p > 0.10$) by contraceptive method (Tables II and IV).

Comment

Despite the increasing number of studies which demonstrate an association between the use of IUDs and PID, few investigators have evaluated the relationship between IUD use and TOA, one of the most serious sequelae of PID.

Some have stated that unilateral TOA is more likely to occur among IUD users than among nonusers who have PID. For example, Golde and associates[2] reported that among 85 women with surgically confirmed TOAs the women were using IUDs in 32 cases. The incidence of unilateral TOA among the IUD users was 62.5% compared with only 32.1% among the 53 women who were not using IUDs. In contrast to the study of Golde and associates, the present investigation indicated similar rates of unilateral TOAs among all patients with an abscess regardless of the type of contraceptive used at the time of diagnosis. This was true whether the diagnosis of TOA was confirmed surgically or was made on the basis of physical examination only. Thus, the concept that IUD use may be more likely to lead to a unilateral TOA was not confirmed in this study.

It is unclear whether surgical management of PID is more frequently required among IUD users than among nonusers. In the present study, women who had IUD-associated pelvic or tuboovarian abscess less frequently required surgical treatment than those who had non–IUD-associated abscesses. The IUD group, on the other hand, had a lower proportion of patients with a prior history of PID and had a higher rate of acute pelvic inflammatory disease than the women using other contraceptive methods. It remains unclear whether or not the use of IUDs leads to a more acute form of PID or whether the presentation of illness (acute or chronic) is more closely associated with a woman's past history. If IUD use does, in fact, lead to a more acute presentation of PID, then one may expect a higher proportion of IUD users, compared to nonusers, to be hospitalized for treatment even if the overall incidence of PID is similar for both groups. If this is true, then studies which evaluate the relative risks of PID for users of different contraceptive methods based only on hospital populations would be expected to report a higher risk of PID for the IUD users. In order to evaluate this possibility, further studies of the association of PID and IUD use, based on nonhospital populations, should be conducted. The higher rate of acute PID and the lower proportion of patients who were surgically treated in the IUD group do not indicate that the PID in this group of patients represents postinsertion endometritis or endoparametritis. Only two of the 67 women using IUDs had the IUDs inserted within 2 weeks of the diagnosis of TOA.

As in any retrospective study with hospital records, there are limitations to the present study. The criteria for diagnosis of TOA were not necessarily uniform, although for cases where the diagnosis was confirmed at surgery, diagnostic errors seem unlikely. Since the management of TOA in terms of the need for surgery and the type of surgery depends upon the individual opinion of the attending physician responsible for the patient's care, differences in rates of surgical treatment for the different groups under study may be related to factors which were not studied in the present investigation.

On the basis of our previous review of the literature on IUDs[1] and on the basis of the present study, it is apparent that further studies will be required to: (1) define more completely the relationship between IUD use and the risk of PID, especially the risk of developing either unilateral or bilateral TOAs; (2) evaluate the need for surgical intervention in IUD-associated PID as opposed to non–IUD-associated PID; and (3) determine whether IUD use compared to use of other contraceptive methods is associated with more virulent forms of PID.

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Utility of assay of alpha subunit of human chorionic gonadotropin in management of gestational trophoblastic malignancies

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All patients who were treated with chemotherapy for malignant gestational trophoblastic neoplasms at the Southeastern Regional Trophoblastic Disease Center and who experienced remission during the period January 1, 1978, through December 31, 1979, were studied in an attempt to determine if the application of an assay for the alpha subunit of human chorionic gonadotropin (hCG-α) could predict those patients who would have recurrences. For each patient, the first three weekly serum samples with undetectable hCG by the hCG β-subunit radioimmunoassay as well as the two preceding samples with detectable hCG were assayed in a homologous hCG-α assay. Results reaffirm previous data that the latter assay is useful only in patients receiving oral contraceptives for suppression of pituitary gonadotropin secretion. No patient who has remained in remission had a mean level of hCG-α in the three hCG-negative samples greater than 1 ng/ml. Of those patients who had recurrences of trophoblastic disease, two of three had hCG-α levels greater than 1.5 ng/ml. Although these data are preliminary, they suggest that the routine measurement of hCG-α in patients successfully treated for trophoblastic disease may aid in identification of a group of patients who require additional or more intensive follow-up and/or additional chemotherapy to prevent later recurrences. (AM. J. OBSTET. GYNECOL. 138:545, 1980.)

A SMALL but important percentage of patients who apparently have been successfully treated for gestational trophoblastic neoplasms have recurrences after remission as evidenced by both an absence of clinical signs of disease and by no detectable human chorionic gonadotropin (hCG) or its beta subunit (hCG-β) on repetitive assay.[1] Since there is secretion of both the α subunit of hCG (hCG-α) and native hCG in a variety of physiologic[2] and pathologic[3] conditions, measurements of hCG-α might be useful in the management of patients receiving treatment for trophoblastic malignancies.

Some authors have suggested that measurement of hCG-α at a time when hCG titers are still elevated may be of value in predicting response to chemotherapy.[3] A recent report from this Center has suggested that patients who had recurrences of trophoblastic neoplasms have an increased detectability of hCG-α while in remission when compared with both patients who did not have recurrences and normal control subjects.[4]

This study was designed to examine further whether the assay for hCG-α is useful in identifying patients at high risk for recurrence who should receive closer follow-up and/or additional chemotherapy.

Material and methods

Patient population. All patients treated with chemotherapy for malignant gestational trophoblastic disease at the Southeastern Regional Trophoblastic Disease Center who experienced remission during the period January 1, 1978, through December 31, 1979, formed the study population. Remission was defined as a min-

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This work was supported by Grant 1-R18-CA19-272 from the National Cancer Institute.

Received for publication June 20, 1980.

Accepted July 7, 1980.

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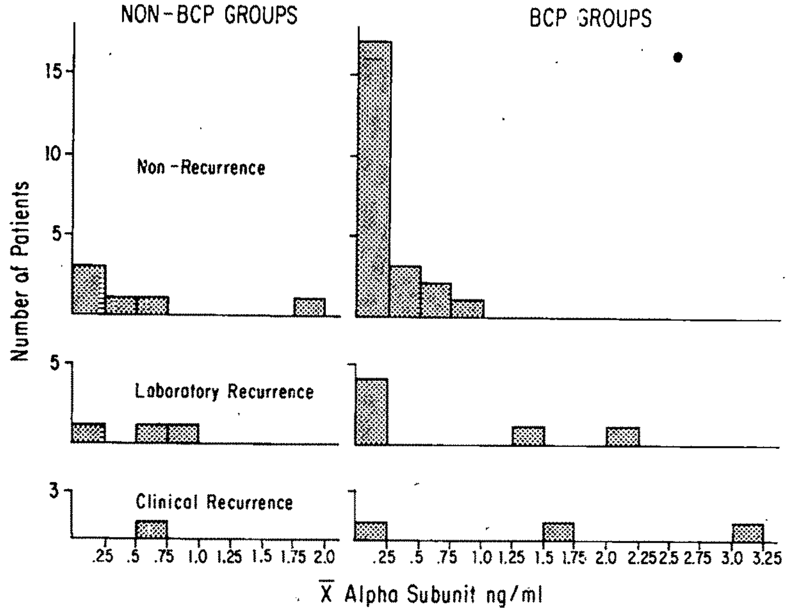

Fig. 1. Representation of the detection and levels of mean hCG-α concentrations in the first three samples negative for hCG in each of the patient groups. Values of hCG-α < 0.25 ng/ml are below the sensitivity of the assay.

Table I. Subdivisions of patient population

	Laboratory recurrence	*Clinical recurrence*	*Non-recurrence*	*Total*
BCP	6	3	23	32
Non-BCP	3	1	6	10
Total	9	4	29	42

imum of three consecutive weekly serum samples with hCG levels less than 5 mIU/ml (by the hCG-β radioimmunoassay)[5] and no clinical evidence of persistent or metastatic disease.[6] Forty-two patients met these criteria and were subdivided into two groups based on whether or not they were using combination oral contraceptives for suppression of pituitary gonadotropin production. There were 32 patients using combination contraceptives (BCP group) and 10 patients not using these compounds (non-BCP group). Each of these groups was further subdivided into laboratory recurrence, clinical recurrence, and nonrecurrence groups. Laboratory recurrence was defined as detectable hCG (>5 mIU/ml) on one or more occasions after remission (as defined above) without clinical signs of disease. These patients may or may not have received additional chemotherapy based on hCG titer alone. Clinical recurrence was defined as hCG detection (>5 mIU/ml) after remission with clinical and/or histologic evidence of disease. All these patients received additional chemotherapy. Nonrecurrence was defined as continued nondetectability of hCG and no clinical evidence of recurrent disease. Table I indicates the number of patients in each group. The characteristics of the patient groups are listed in Table II. There were no significant differences among groups in age, parity, preceding pregnancy type, good or poor prognostic signs,[7] the presence or site of metastases, and use of oral contraceptives.

Serum samples. Patients receiving chemotherapy had serum samples drawn weekly for hCG-β radioimmunoassay. After assaying for hCG, samples were stored at −20° C. The first three consecutive samples with undetectable hCG as well as the preceding two samples with detectable hCG from each patient were selected for assay for hCG-α (Fig. 1). Assays were not performed until after the patient's discharge, and results were not returned to the patient's chart, with the results thus playing no role in the clinical management of the patient.

Assay. hCG-α was determined with a homologous hCG-α assay as previously described[4] with highly purified hCG-α and anti–hCG-α provided by the National Institute of Arthritis, Metabolism, and Digestive Diseases and the National Institute of Child Health and Human Development. The within-assay and between-assay coefficients of variation were both 5.1%, as determined by the methodology of Rodbard.[8] The minimum sensitivity of the assay was 0.25 ng/ml.

Results

Non-BCP groups. Comparisons between the hCG values (in milli-International Units per milliliter from the hCG-β assay) and the hCG-α values in the last two serum samples with detectable hCG before the three negative samples revealed a lack of any statistically significant linear ($r = 0.304$) or other correlations. In all calculations and comparisons, undetectable values of hCG-α were assumed to have their maximum possible value, 0.25 ng/ml.

For each patient, a mean hCG-α value was determined from the hCG-α values for the three hCG-negative samples. These mean values are listed in Table III for each patient in those groups not using oral contraceptives. There were no statistically significant differences among these three groups by chi-square or analysis of variance (ANOVA). In those patients in whom hCG-α was detected (>0.25 ng/ml) there were also no significant differences among groups.

BCP groups. Comparisons between the hCG values and the hCG-α values in the last two serum samples with detectable hCG before the first three negative values in the BCP groups also revealed a lack of any significant linear ($r = 0.160$) or other correlations.

The mean hCG-α value for each patient in the BCP groups (calculated as in the preceding section) is listed in Table IV. Because of the large number of undetectable values in these groups, the standard parametric statistical tests were not applied to these data. With the use of chi-square analysis based on detectability or nondetectability of hCG-α, there were no statistically significant differences among these groups when all observations were included. When only those patients in whom hCG-α was detected were considered, however, analysis of variance revealed a significant difference between the nonrecurrence group and the laboratory recurrence and clinical recurrence groups ($F = 12.05$, $P < 0.01$). There was no statistical difference between the laboratory recurrence and clinical recurrence patients with detectable hCG-α.

Table II. Characterization of patient population

	Nonrecurrence	*Laboratory recurrence*	*Clinical recurrence*
No. in group	29	9	4
Age range	16-50	17-42	26-29
Parity range	0-8	0-3	1-3
Metastases	14 (48.3%)	3 (33.3%)	3 (75%)
Preceding pregnancy:			
Mole	24 (82.8%)	8 (88.9%)	2 (50%)
Term	3 (10.3%)	1 (11.1%)	2 (50%)
Ectopic	1 (3.4%)	–	–
Induced abortion	1 (3.4%)	–	–
Poor prognosis	8 (27.6%)	1 (11.1%)	4 (100%)
Received oral contraceptives	23 (79.3%)	6 (66.7%)	3 (75%)

Table III. Mean hCG-α in the first three negative samples for non-BCP groups

Nonrecurrence (ng/ml)	*Laboratory recurrence (ng/ml)*	*Clinical recurrence (ng/ml)*
<0.25 (3 patients)	<0.25 (1 patient)	0.52
0.48	0.63	–
0.51	0.99	–
1.92	–	–

Table IV. Mean hCG-α in the first three negative samples for BCP groups

Nonrecurrence (ng/ml)	*Laboratory recurrence (ng/ml)*	*Clinical recurrence (ng/ml)*
<0.25 (17 patients)	<0.25 (4 patients)	<0.25 (1 patient)
0.27	1.42	1.50
0.32	2.10	3.13
0.34	–	–
0.50	–	–
0.65	–	–
0.80	–	–

Comment

The alpha subunit is not unique to hCG but is nearly identical among hCG, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Moreover, the alpha-subunit assay, in addition to measuring free alpha subunit from trophoblastic and pituitary sources, will also measure, to a degree, circulating native TSH, LH, and hCG.[9]

The absence of significant differences among groups in those patients not using oral contraceptives further substantiates our earlier observation that measurement of alpha subunit is not useful in patients unless they are receiving pituitary suppressive therapy (BCP).[4] Combination oral contraceptives have been shown to decrease circulating levels of LH and FSH (and to greatly diminish or abolish the midcycle LH surge).[10] Since a major portion of the immunoreactive alpha subunit represents immunologic recognition of the circulating pituitary gonadotropins,[9] reducing their circulating concentrations increases the specificity of the alpha-subunit assay.

The lack of any significant correlation between the alpha subunit and native hCG as measured by the hCG-β assay suggests that in patients with trophoblastic neoplasms these two assays measure products which reflect different tumor secretory activities. This is especially true in patients using combination oral contraceptives, since their lower levels of circulating gonadotropins increase the specificity of the assay for the alpha subunit of trophoblastic origin.

The current study, as well as a previous report,[11] does not indicate any advantage to measurement of the alpha subunit at times when circulating hCG is still present because of the immunologic recognition of the circulating hCG by the anti–hCG-α antibody. After hCG becomes undetectable, the lack of detectable alpha subunit does not provide a clear indication that the patient will not have a recurrence, although a higher percentage of patients in the nonrecurrence groups had undetectable alpha subunit (73.9%) than either the laboratory recurrence (66.7%) or clinical recurrence (33.3%) groups. While the follow-up of the nonrecurrence groups is limited (6 to 30 months), the previously reported chances of recurrence are less than 2% after 3 months and 1% after 12 months of remission.[12]

The significance of low-level elevations of alpha subunit (mean < 1 ng/ml) in the first three negative samples of patients using oral contraceptives is unclear. Six patients in the nonrecurrence group (26.1%) had mean alpha-subunit values in this range while no patients in either of the recurrence groups had similar low elevations of alpha subunit. If low-level elevations of alpha subunit are found, it would be prudent to manage these patients with more frequent hCG-α and hCG-β assays than usually recommended.[6]

If high levels of alpha subunit (mean ≥ 1.5 ng/ml) are found in the first three negative samples, our data, although limited, suggest that there is a high risk of recurrent trophoblastic neoplasms and that consideration should be given to additional chemotherapy in this group of patients. Two of four patients in the current study with high levels of alpha subunit during early remission had subsequent clinical recurrences. All patients in this study received at least one additional course of chemotherapy after the first negative hCG titer; some of these patients received additional courses of chemotherapy after three negative titers. The effect of this additional chemotherapy on the levels or detectability of alpha subunit, as well as on the chances of recurrence, is unknown.

The significance of the laboratory recurrence group is unclear. Of the six patients using oral contraceptives in this group, two had high levels of alpha subunit. One of these patients received additional chemotherapy which may have prevented clinical evidence of recurrent disease. The other patient may have had recurrent disease with "spontaneous" remission on a delayed effect of previous chemotherapy on residual tumor, or she may have been disease free with the apparent elevation merely reflecting cross-reactivity with circulating gonadotropins or TSH.

At the present time, our conclusions remain preliminary as the number of patients studied is small and the period of follow-up is short. However, the finding of relatively high levels of alpha subunit in two thirds of our patients who had clinical recurrences as well as the absence of high levels of alpha subunit in those patients who have remained in remission suggests that the alpha-subunit assay should play a role in the routine management of patients with trophoblastic malignancies. The detection of alpha subunit after successful chemotherapy may indicate a group of patients at high risk for recurrence and who require closer follow-up and/or additional chemotherapy to achieve sustained remission.

The anti–hCG-α antibody and highly purified hCG-α were provided by the NICHHD and NIAMDD. We acknowledge the excellent technical assistance of Ms. Mary Lou Everett.

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Analysis of factors contributing to treatment failures in Stages IB and IIA carcinoma of the cervix

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Between April, 1971, and September, 1977, 98 patients with Stages IB and IIA cervical cancer who underwent surgical exploration prior to treatment at Hershey Medical Center were studied. Those who had bulky primary tumor (≥4 cm) had a higher incidence of nodal metastases (80% vs. 16%), local recurrences (40% vs. 5%), and distant metastases (40% vs. 1%). Patients who had positive nodes had more local recurrences (24% vs. 6%) and distant metastases (28% vs. 0%). Those with grossly positive nodes had more distant metastases (60% vs. 7%) than those with microscopically positive nodes. Those who had positive nodes, vascular invasion, and/or deep invasion of the cervix (≥70% of thickness) in the radical hysterectomy specimen had more nodal metastases and local recurrences. Postoperative radiation seemed to prevent local recurrences (40% vs. 6%) and improve the 2-year tumor-free survival rate (94% vs. 55%). Patients who had bulky primary tumors and/or grossly positive nodes at laparotomy may require systemic therapy in view of the high incidence of distant failures. (AM. J. OBSTET. GYNECOL. 138:550, 1980.)

SUCCESSFUL treatment of Stages IB and IIA cancer of the cervix requires control of the central tumor and eradication of metastatic cancer foci when present in the regional nodes.

The purpose of this investigation was to determine the prognostic significance of pelvic node metastasis, size and depth of invasion of the primary lesion, and the presence of vascular invasion in the radical hysterectomy specimen. The value of postoperative radiotherapy for those in the high-risk group was also evaluated.

Methods

One hundred forty patients with previously untreated FIGO (International Federation of Gynaecology and Obstetrics) Stages IB and IIA carcinoma of the cervix were treated at the Milton S. Hershey Medical Center of The Pennsylvania State University between April, 1971, and September, 1977. Forty-two of these patients who were medically ineligible or had barrel-shaped cervix underwent no surgical exploration and were not included in the study, thus leaving as eligible a total of 98 patients who underwent laparotomy.

The patients were divided into three groups according to the treatment plan (Table I). At laparotomy, sampling of aortic nodes and multiple biopsies of grossly enlarged pelvic nodes were done. If nodes were positive and/or the primary tumor was found to be more advanced, the condition was regarded as nonresectable and the patient was treated with radiotherapy alone. If nodes were negative, radical hysterectomy with bilateral pelvic lymphadenectomy was performed. Postoperative radiation was usually administered to

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Presented at the Sixty-fifth Annual Meeting of the Radiologic Society of North America, Atlanta, Georgia, November 29, 1979.

Received for publication February 13, 1980.

Revised June 27, 1980.

Accepted July 21, 1980.

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Table I. Ninety-eight patients with Stages IB and IIA cervical cancer who underwent laparotomy

Treatment	No. of patients		Average age (yr)
	Stage IB	Stage IIA	
Operation*	65	8	45
Operation plus postoperative radiotherapy†	11	1	46
Radiotherapy	9	4	47
Total	85	13	

*Radical hysterectomy with bilateral pelvic lymphadenectomy.

†Radical hysterectomy with bilateral pelvic lymphadenectomy plus postoperative radiotherapy.

Table II. Sites of lymph node metastases* (Stages IB and IIA cervical cancer)

Lymph node group	Right	Left	Both	Total†	
				No.	%
External iliac	4	5	2	11	36
Obturator	4	2	0	6	19
Hypogastric	2	3	0	5	16
Parametrial	2	1	0	3	9
Total pelvic	12	11	2	25	80
Common iliac	2	1	2	3	10
Para-aortic	1	2	0	3	10
	15	14	2	31	100

*Number of lymph nodes dissected: eight to 76 nodes (average, 24.3).

†Number of sites of nodal metastases.

Table III. Incidence of lymph node metastases

Characteristics	(+) Nodes	
	No.	%
Stage:		
IB	21/85	25
IIA	4/13	31
Histologic features:		
Squamous cell carcinoma	23/88	26
Adenocarcinoma	2/8	25
Adenosquamous carcinoma	0/2	0
Size of primary tumor:*		
≥4 cm	12/15	80†
<4 cm	13/83	16
Depth of invasion‡:		
≥70% thickness	6/15	40†
<70% thickness	9/70	13
Vascular invasion‡:		
+	5/8	63†
−	10/77	13

*Clinical measurement.

†$P < 0.05$ (chi-square test).

‡Radical hysterectomy specimen findings.

those in whom positive nodes were found on radical hysterectomy and pelvic lymphadenectomy specimen.

A total of 73 patients underwent operation alone (radical hysterectomy with bilateral pelvic lymphadenectomy). Twelve patients underwent operation plus postoperative radiotherapy: 10 patients with positive nodes, one patient with positive vaginal resection margin, and one patient in whom the tumor penetrated through the wall on the radical hysterectomy specimen. (The other five patients with positive nodes did not receive postoperative radiotherapy because of surgical complications.) Thirteen patients underwent radiotherapy alone: nine patients with gross nodal metastases and four patients with more advanced disease at laparotomy (the tumor extended into the parametrium, 1, pelvic wall, 1, and bladder, 2).

Radiotherapy was administered in a fairly standard fashion and it consisted of external-beam radiotherapy to the whole pelvis with the use of a 10-mev linear accelerator followed by intracavitary brachytherapy. External-beam radiation therapy delivered a minimum whole-pelvic dose of 5,100 rads through anterior-posterior opposed or four-field technique. All fields were treated every day, 5 days a week with a daily tumor dose of 170 rads. The minimum treatment volume was maintained as follows: superiorly, the upper margin of L5; inferiorly, the upper third of the obturator foramen, or lower if indicated; laterally, at least 1 cm beyond the lateral margin of the bony pelvis at the widest plane of the pelvis. Additional para-aortic node irradiation was given to three patients with positive para-aortic nodes. Intracavitary brachytherapy consisted of the Fletcher-Suit afterloading system with cesium-137. Two 48-hour applications with a 2-week interval were used for primary radiotherapy; the first insertion of cesium delivered an average of 2,714 (2,587 to 3,226) mg hr, with 2,234 rads (2,088 to 2,640) to point A, and 486 rads (336 to 576) to the lateral pelvic wall (lateral margin of the bony pelvis at the widest plane through the pelvis). The second insertion delivered an average of 2,964 (2,145 to 3,144) mg hr with 2,382 rads (1,914 to 2,640) to point A and 434 rads (384 to 528) to the lateral pelvic wall. Therefore, a total of 5,679 mg hr with 4,616 rads to point A and 920 rads to the lateral pelvic wall was delivered in two insertions. For the postoperative radiotherapy group, seven patients underwent external pelvic irradiation only (5,100 rads in 6 weeks), and five patients underwent, in addition, one 48-hour insertion of a vaginal applicator to deliver an average of 1,566 (960 to 1,920) mg hr with 4,864 (4,080 to 5,760) rads to the vaginal surface.

The size of the primary tumor, as measured at the time of initial clinical staging, was divided into two

Table IV. Sites of failure by treatment modalities

Treatment	No. of patients	Local failures				Distant metastases	
		Central		Lateral			
		No.	%	No.	%	No.	%
Operation	73	2	3	3	4	0	0
Operation plus postoperative radiotherapy	12	1	8	0	0	1	8
Radiotherapy	13	1	13	3	23	6	46
Total	98	4	4	6	6	7	7

Table V. Treatment results

	No. of patients	Local recurrence		Distant metastasis		2-Year survival* (NED)†	
		No.	%	No.	%	No.	%
Stage							
IB	85	9	11	5	6	72	85
IIA	13	1	8	2	15	11	85
Histologic features:							
Squamous-cell carcinoma	88	8	9	7	8	75	85
Adenocarcinoma	8	1	13	0	0	7	88
Adenosquamous carcinoma	2	1	50	0	0	1	50
Size of primary tumor							
≥4 cm	15	6	40	6	40	7	47‡
<4 cm	83	4	5	1	1	76	92
Depth of invasion							
≥70% thickness	15	4	27	2	13	10	67‡
<70% thickness	70	3	4	0	0	65	93
Vascular invasion							
+	8	2	25	1	13	4	50‡
−	77	4	5	0	0	72	94

*Absolute recurrence-free survival.

†No evidence of disease at the follow-up examination immediately after the cut-off time at 24 months after treatment.

‡$P < 0.05$.

groups: 4 cm or more vs. less than 4 cm on clinical measurement. The hysterectomy specimen was examined in a fresh state and after formalin fixation. Full-thickness sections through the tumor (minimum of three) were taken from areas grossly showing the greatest depth of penetration. These were stained with hematoxylin and eosin. The maximum depth of tumor invasion was determined as a percentage of thickness of the cervical-endocervical segment. Vascular invasion (lymphatic and venous) was determined from the slides stained with hematoxylin and eosin. Sections of all lymph nodes were studied. When nodes were large, a single representative section was taken. In specimens in which nodes were not grossly detectable, a sample of tissue was subjected to search for foci of lymphatic tissue. The incidence of nodal metastasis was presented according to size of primary tumor, degree of depth of invasion, and presence of vascular invasion.

Treatment results were analyzed as local recurrence, distant metastases, and 2-year absolute recurrence-free survival (NED), by size of primary tumor, status of lymph node metastases, hysterectomy specimen findings (vascular invasion and depth of invasion), and treatment modalities. Particularly, treatment outcome was compared between operation alone and operation plus postoperative radiotherapy in those in the high-risk group with lymph node metastases, deep penetration of the tumor, and vascular invasion. Finally, complications were analyzed by each treatment modality in order to find out whether the combined-therapy group had increased complications. Follow-up care was carried out in the Gynecologic Oncology Clinic. Absolute survival was calculated from the initial date of treatment. All patients were followed until death or for a minimum of 2 years from the date of first treatment. Three patients were lost to follow-up and counted as dead. Statistical evaluation was carried out according to the chi-square method.

Results

Of a total of 98 patients, 10 (10%) had gross lymph node metastases at the time of laparotomy; 15 of 85

Table VI. Status of nodal metastases and prognoses

Nodal site	*Status of metastasis*	*Local recurrence*	*Distant metastases*	*2-Year survival (NED)*
Pelvic	Gross* (+)	0/6	3/6	4/6
	Micro† (+)	3/13	0/13	9/13
		3/19 (16%)	3/19 (16%)	13/19 (68%)
Common iliac	Gross (+)	1/2	1/2	1/2
	Micro (+)	0/1	0/1	1/1
		1/3 (33%)	1/3 (33%)	2/3 (67%)
Para-aortic	Gross (+)	2/2	2/2	0/2
	Micro (+)	0/1	1/1	1/1
		2/3 (67%)	3/3 (100%)	1/3 (33%)
	Gross (+)	3/10 (30%)	6/10 (60%)	5/10 (50%)‡
	Micro (+)	3/15 (20%)	1/15 (7%)	11/15 (73%)
Total	Node (+)	6/25 (24%)	7/25 (28%)	16/25 (64%)§
	Node (−)	4/73 (6%)	0/73 (0%)	67/73 (92%)

*Gross nodal metastases at laparotomy, which were all removed.

†Microscopically positive nodes on radical hysterectomy specimen.

‡Not significant.

§$P < 0.05$.

Table VII. Retrospective comparison of treatment results in the high-risk group: Operation alone vs. operation plus postoperative radiotherapy

	Local recurrence				*2-Year survival (NED)*			
	*Operation**		*Operation* + RT†*		*Operation**		*Operation + RT†*	
Pathologic specimen findings	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Lymph node metastasis (+)	2/5‡	40	1/10	10	2/5	40	9/10	90
Depth of invasion (≥70% thickness)	4/10	40	0/5	0	7/10	70	5/5	100
Vascular invasion (+)	2/5	40	0/3	0	2/5	40	3/3	100
Total	8/20	40	1/18	6	11/20	55	17/18§	94

*Radical hysterectomy.

†Radical hysterectomy plus postoperative radiotherapy.

‡Five patients who had microscopic positive nodes did not receive postoperative radiotherapy because of surgical complications.

§$P = 0.05$.

patients (18%) who underwent radical hysterectomy with bilateral pelvic lymphadenectomy were found to have unexpected lymph node metastases microscopically in the pathologic specimen. Of the 85 patients with Stage IB carcinoma, 21 had positive nodes (25%); 19 had pelvic only, one had pelvic and para-aortic, and one had para-aortic only. Of the 13 patients with Stage IIA carcinoma, 4 patients had nodal metastasis (31%), three had pelvic only, and one had para-aortic only. Table II shows the distribution of lymph node metastases; the external iliac node was the most common site of involvement. Only two patients had lymph node metastases bilaterally.

The incidences of lymph node metastasis, by stage, histologic features, size of primary tumor, as well as depth of invasion and vascular invasion, are shown in Table III. The stage (IB vs. IIA) and histologic features (squamous-cell carcinoma vs. adenocarcinoma) do not seem to influence lymph node metastasis; however, patients with bulky primary tumors (≥4 cm) had a significantly higher incidence of nodal metastasis than did those with smaller tumors (80% vs. 16%). Those who had vascular invasion and/or deep invasion of the cervix (≥70% of thickness) on hysterectomy specimen had more lymph node metastases.

In this series, 88% of recurrence occurred within 24 months, and all within 30 months of treatment. Therefore, the 2-year NED rate is reliable for the purpose of comparing the various treatment results. Ten patients experienced local failure in the pelvis (four central and six lateral), and seven patients developed distant metastases (four patients in the bones, two patients in the supraclavicular nodes, and one patient in the mediastinum). The sites of failure by treatment modality are shown in Table IV. Since patients with the more unfavorable conditions received radiotherapy alone, the

Table VIII. Complications by treatment modalities

Complications	*Operation (73 patients)*	*Operation + postoperative radiotherapy (12 patients)*	*Radiotherapy (13 patients)*
Fistulas	7 (9%)*	0 (0%)	1 (7%)†
Urinary retention and/or incontinence	13	1	0
Lymphocyst	2	0	0
Pelvic abscess	2	0	0
Acute pyelonephritis	1	0	0
Surgical injury to bladder or rectum	3	1	0
Severe hemorrhage	1	0	0
Small bowel obstruction	1	0	1
Rectosigmoiditis	0	0	1
Severe complication‡	17 (23%)	1 (8%)	3 (23%)

*Seven patients with 10 fistulas (five ureterovaginal, three vesicovaginal, one rectovaginal, and one vesicocutaneous) underwent surgical correction within 2 to 10 months after radical hysterectomy, with good primary results in all cases.

†Rectovaginal fistula.

‡Required second surgical intervention.

Table IX. Two-year absolute survival (NED)

	Stage IB cancer			*Stage IIA cancer*		
	No.	*NED*(%)*	*Overall survival (%)*	*No.*	*NED*(%)*	*Overall survival (%)*
Operation	58/65	89	95	7/8	88	100
Operation plus postoperative radiotherapy	10/11	91	91	1/1	100	100
Radiotherapy	4/9	44	67	3/4	75	75
Positive nodes	14/21	67	81	2/4	50	75
Negative nodes	58/64	91	95	9/9	100	100
Total	72/83	85	92	11/13	85	92

*Recurrence-free survival.

treatment results were worse in that group.

The incidences of local recurrence, distant metastasis, and absolute 2-year survival (NED) rate by stage, histologic findings, and size of primary tumor, as well as depth of invasion and vascular invasion, are shown in Table V. The stage and histologic features did not seem to influence the prognosis, but the patients who had bulky primary tumors had a higher incidence of local recurrence and distant metastases and a lower 2-year NED rate, and those who had vascular invasion and/or deep invasion of the cervix had a higher incidence of local recurrence and lower 2-year NED rate. The correlations of the status of nodal metastases with prognosis are shown in Table VI. The patients who had positive nodes had more local recurrences, distant metastases, and lower 2-year NED rates. The patients with grossly positive nodes had more distant metastases than those with only microscopically positive nodes. All three patients with para-aortic node metastases developed distant metastases.

A retrospective analysis comparing the treatment results in the high-risk group for patients who received postoperative radiotherapy and for those who did not revealed that postoperative radiotherapy seemed to prevent local recurrence and improve the 2-year NED rate (Table VII).

The complications by treatment modality are shown in Table VIII. The complication rate in those patients with the combined approach was not increased in this small series; one patient had occasional urinary incontinence, the other patient sustained surgical injury to the bladder which was repaired. The severe complications which required a second surgical intervention in each treatment group were comparable.

The 2-year absolute survival (NED) rate by stage, treatment, and nodal status is shown in Table IX. Surgical procedures for resectable lesions achieved an 89% 2-year NED rate, and postoperative radiotherapy a 92% rate, whereas primary radiotherapy for nonresectable lesions achieved a 54% 2-year NED rate. The 2-year NED rates for patients who had positive nodes and for those who had negative nodes were 64% and 92%, respectively.

Of the five patients who experienced surgical failure, four underwent radiotherapy, and success was achieved in three. One patient with local recurrence in the postoperative radiotherapy group underwent chemotherapy which was not successful. Of four patients who experienced radiotherapy failure, one underwent a successful operation (total pelvic exenteration) and three received chemotherapy that failed.

Comment

It is evident from this retrospective analysis that in patients with Stages IB and IIA cervical cancer the various parameters associated with the prognosis are the size of the cervical lesion, lymph node metastases, vascular invasion, and depth of invasion of the tumor. Piver and Chung[6] and Freidell and Graham[3] have confirmed that there is good correlation between the size of the cervical tumor, lymph node metastases, and survival. The patients with bulky primary tumors (≥4 cm) had a higher incidence of failure (40% with both local recurrence and distant metastasis) in our series. Those patients with barrel-shaped cervices did not undergo laparotomy and were treated with either primary radiotherapy or preoperative radiotherapy followed by simple hysterectomy. Therefore, these patients were not included in this study.

The patients with vascular invasion had more nodal metastases and local recurrences than did those without vascular invasion. In Van Nagell and associates[7] series of 100 patients with Stage IB cervical cancer who underwent radical hysterectomy, vascular invasion was associated with a significant increase in nodal metastases and tumor recurrences. No reports have previously been made of the prognostic significance of depth of invasion of cervical cancer. In the present series, the patients with deep invasion of the tumor (≥70% of thickness) had three times as many nodal metastases and seven times as many local recurrences as those who did not have deep invasion. Boyce and associates[1] also noticed that there was good correlation between the depth of invasion, vascular invasion, and prognosis in Stage IB cervical carcinoma.

In treatment by radiation or surgical procedures, the lymph nodes are more of a problem than is the central disease. Several authors have reported 5-year survival rates as high as 61%[5] and as low as 38%[2] in patients with Stage IB carcinoma who at the time of radical hysterectomy and bilateral pelvic lymphadenectomy had pelvic nodes containing metastatic cancer. There are a few reports of survival of patients with operation-documented positive nodes who were treated by radiotherapy. Of 13 patients with positive nodes surgically documented prior to radiation, Lagasse and associates[4] reported that only three survived 5 years (23%). Wharton and associates[8] reported that 11 of 64 patients with positive nodes were surviving 13 to 38 months (17%). In the present series, the 2-year NED rate for patients with positive nodes was 64%; those who had lymph nodes that contained microscopic deposits of cancer achieved only a 73% 2-year NED rate, compared to a 50% rate for those with grossly positive nodes at laparotomy. The latter group had nine times more distant metastases than the former group. Also, the site of lymph node metastases seemed to affect the incidence of distant metastases, since 67% of those with common iliac and/or para-aortic node involvement developed distant metastases, whereas only 16% of those with only pelvic node metastases developed distant metastasis.

The role of postoperative radiotherapy in the management of cervical carcinoma has not been extensively studied. In this series, postoperative radiotherapy for those with vascular invasion and/or deep invasion of the cervix and for those with microscopically involved nodes seemed to prevent local recurrence and improve the 2-year tumor-free survival rate. The 2-year NED rates for operation alone and for operation plus postoperative radiotherapy in the high-risk group were 55% and 94%, respectively. The difference was significant at $P = 0.05$. Since the complication rate in those patients treated with the combined approach was not increased, our early observations suggest, therefore, that postoperative radiotherapy is indicated in patients who have microscopically positive nodes, vascular invasion, and/or deep penetration of tumor in the radical hysterectomy specimen. This remains to be proved by randomized prospective study. In addition, if further studies confirm the risk of disseminated disease in those patients with gross metastases to lymph nodes at laparotomy, adjuvant chemotherapy or immunotherapy in addition to local irradiation should be considered in the future.

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The significance of residual disease after radiotherapy in endometrial carcinoma: Clinicopathologic correlation

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The extent and location of residual disease after radiotherapy in 91 patients with early invasive endometrial carcinoma was related to tumor grade, clinical stage, and outcome. In 20% the grade (according to the International Federation of Gynecology and Obstetrics) differed in curettage and hysterectomy specimens. In at least 12% the clinical stage was unreliable. Death from endometrial carcinoma was significantly more frequent in patients with residual disease after radiotherapy than in those without residual disease. The poorest prognostic features were blood vessel invasion in the myometrium, irrespective of depth of invasion, and residual disease outside the uterus. Treatment of endometrial carcinoma should be based on accurate knowledge of prognostic features determined by initial surgery. (AM. J. OBSTET. GYNECOL. 138:557, 1980.)

SINCE CARCINOMA of the endometrium is now the most common gynecologic malignancy in the United States,[1] it is imperative that therapeutic methods be reassessed to determine the most effective treatment protocols. While there is clear evidence that radiation therapy has a role in the management of endometrial carcinoma, including the reduction of vaginal recurrences,[2] the method and timing of radiation therapy remain controversial.

Many reports have indicated that anaplastic, undifferentiated, or grade 3 (according to the International Federation of Gynecology and Obstetrics) lesions carry poor prognoses.[3-5] Poor outcomes are associated with extensive disease found at hysterectomy, especially deep myometrial invasion, extension to the cervix, and extrauterine extension.[4-6]

The purpose of this study was to compare the histologic characteristics and extent of disease determined by curettage and clinical examination with findings at operation, with and without preoperative radiation therapy, and to correlate this information with outcome.

Material and methods

The records of all patients with histologic diagnoses of carcinoma of the endometrium in the Kings County Hospital–State University Hospital Tumor Registry from 1951 to 1976 were reviewed. Patients whose diagnoses were established by fractional curettage, whose disease was staged clinically as I or II, and whose curettage and hysterectomy specimens were completely available for review were included in this study. Prior to 1971, the FIGO clinical stage[7] was determined by chart review with particular emphasis on initial physical examination, laboratory findings, operative description, and histologic report.

Treatment. Two treatment methods were identified: (1) radiotherapy followed by operation and (2) operation followed by radiation therapy. In the initial radiotherapy method, Stage I patients had intracavitary radium delivered by Heyman capsules when the

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Received for publication December 19, 1979.

Accepted June 30, 1980.

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Table I. Histologic grade at curettage and hysterectomy

		Patients with disease at hysterectomy				
			Hysterectomy specimen			
Grade	*All patients*	*Curettage specimen*	*Same grade*	*Higher grade*	*Lower grade*	*Percent change*
Preoperative radiotherapy:						
Grade 1	62 (68%)	29	27	2	–	7
Grade 2	22 (24%)	9	4	3	2	56
Grade 3	7 (8%)	6	5	–	1	17
Total	91 (100%)	44	36	5	3	18
Initial operation:						
Grade 1	12 (60%)	10	8	2	–	20
Grade 2	6 (30%)	6	5	–	1	17
Grade 3	2 (10%)	2	1	–	1	50
Total	20 (100%)	18	14	2	2	22

Table II. Histologic type at curettage and hysterectomy

		Patients with disease at hysterectomy			
			Hysterectomy specimen		
Histologic type	*All patients*	*Curettage specimen*	*Same type*	*Other type*	*Percent change*
Preoperative radiotherapy:					
Papillary/glandular	60	29	29	–	–
Adenoacanthoma	24	9	6	3	33
Adenosquamous	2	1	1	–	–
Anaplastic	5	5	5	–	–
Total	91	44	41	3	7
Initial operation:					
Papillary/glandular	12	11	11	–	–
Adenoacanthoma	6	5	5	–	–
Anaplastic	2	2	2	–	–
Total	20	18	18	–	0

uterine cavity exceeded 8 cm and Fletcher Suit applicators when the uterine cavity was less than 8 cm, followed by ovoids to the vagina. The dose from the two intracavitary applications, given 7 to 10 days apart, ranged from 6,000 to 8,000 mg hours. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed 6 weeks after the radiotherapy. Stage II patients initially received external radiation therapy to the midplane of the pelvis via a cobalt-60 machine delivering from 4,000 to 5,000 rads through 15 by 15 cm anteroposterior ports. This was followed immediately by intracavitary radium and operation as for Stage I patients. Patients with extrauterine disease at operation received adjunctive chemotherapy.

In the initial surgery method, all patients had Stage I disease. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were followed in 10 to 21 days by radiotherapy. Before 1976, patients underwent operation first when there was a question of an accompanying pelvic or abdominal mass. In 1976, there was a change in the treatment protocol for Stage I disease. All patients had initial total abdominal hysterectomy and bilateral salpingo-oophorectomy followed by radium via vaginal ovoids delivering the same dose as above. Those with significant myometrial invasion, pelvic extension, and/or grade 3 histology were given additional pelvic radiation as outlined above.

In both treatment methods, pelvic and para-aortic nodes were carefully palpated during laparotomy but were biopsied only if clinically suspicious.

Review of pathology. All curettage and hysterectomy specimens were examined by three pathologists in the following manner.

Diagnostic fractional curettage. The curettings were classified according to the FIGO system into grade 1 lesions exhibiting purely glandular or papillary growth pattern, grade 3 lesions characterized by a predominantly solid or anaplastic carcinomatous component, and grade 2 lesions which contained both. Only solid malignant growth patterns, not benign squamous (acanthomatous) metaplasia, were considered as evidence of higher grade lesions. In each case the accompanying endocervical curettage was examined for the presence of endometrial carcinoma and classified into

Table III. Disease status after radiotherapy by histology

Histology	*No. of cases*	*No residual disease*	*Residual disease*	*Percent with residual disease*
Histologic grade:				
Grade 1	62	33	29	47
Grade 2	22	13	9	41
Grade 3	7	1	6	86*
Total	91	47	44	48
Histologic type:				
Papillary or glandular	60	31	29	48
Adenoacanthoma	24	15	9	38
Adenosquamous	2	1	1	50
Anaplastic	5	–	5	100
Total	91	47	44	48

*There is significantly more residual disease in grade 3 lesions ($p < 0.05$ by Fisher's exact test).

two groups: those with definitive evidence of endometrial carcinoma invading endocervical glands or stroma and those showing separate fragments of endometrial carcinoma mixed with benign endocervical glands and stroma. Both groups were classified as positive for endocervical involvement.

All tumors were also examined for their major histologic growth pattern and classified as either (1) papillary or glandular, (2) adenoacanthoma, (3) adenosquamous, (4) predominantly anaplastic or solid, (5) secretory or mucin-producing, and (6) a combination of these. Adjacent benign endometrial tissue was examined for the presence of cystic or adenomatous hyperplasia, atrophy, and proliferative or secretory changes.

Hysterectomy. Hysterectomy slides were evaluated for residual endometrial carcinoma, the histologic FIGO grade, and the histologic type.

One hundred eleven patients met the criteria. Ninety-one patients received initial radiotherapy followed by hysterectomy, while 20 patients were treated by initial operation, followed by postoperative radiotherapy. Diagnosis was made before 1976 in 13 of the latter group; therefore these patients underwent initial operation because of accompanying abdominal or pelvic mass. Ten had leiomyomas of the uterus, two had ovarian metastases, and one had omental metastases.

Results

Histologic grade and type. We analyzed two treatment methods: preoperative radiotherapy followed by operation and initial operation followed by radiotherapy.

Table IV. Disease status after radiotherapy by stage and grade.

Stage	*Grade*	*No. of cases*	*No residual disease*	*Residual disease*	*Percent with residual disease*
IA	1	36	20	16	44
	2	4	2	2	50
	3	3	–	3	100
	Total	43	22	21	49
IB	1	12	6	6	50
	2	5	3	2	40
	3	1	–	1	100
	Total	18	9	9	50
II	1	14	7	7	50
	2	13	8	5	39
	3	3	1	2	67
	Total	30	16	14	47
Total—all stages		91	47	44	48

Two thirds of each treatment group had FIGO grade I lesions while about 10% had grade 3 lesions (Table I). There was residual disease in the hysterectomy specimen in 44 of the 91 patients (48%) treated with preoperative radiotherapy and in 18 of 20 patients with initial operation (90%). In 82% of those receiving preoperative radiotherapy and 78% of those without preoperative radiation, the histologic grade was the same in the curettage and hysterectomy specimens. The frequencies of higher and lower grades in the hysterectomy specimens were similar in both groups. The grade of the tumor was inaccurate in about 20% of the cases, but there was no evidence that radiation itself affected the grade of the tumor. In analyses that follow, histologic grade of the curettage specimen is used.

In Table II the histologic type of tumor in curettage and hysterectomy specimens is compared. The anaplastic and adenosquamous lesions correspond to those classified as grade 3 in Table I. Three lesions classified as adenoacanthoma in the curettage were classified as glandular in the hysterectomy specimen after radiotherapy (7% change). All other cases had the same histologic type in both curettage and hysterectomy specimens.

Residual disease after radiotherapy.

Histology. Table III shows the disease status after radiotherapy according to histologic grade or type.

Table V. Site of residual disease by grade and stage

Grade or Stage	*Residual tumor in uterus only*				Total with tumor in uterus	Extrauterine disease	Total
	Depth of invasion		*Vascular invasion*				
	<50%	*>50%*	*Absent*	*Present*			
Grade 1	19	6 (24%)*	23	2 (8%)	25	4	29
Grade 2	3	3 (50%)	5	1 (12%)	6	3	9
Grade 3	2	3 (60%)	2	3 (60%)†	5	1	6
Total	24	12 (33%)	30	6 (17%)	36	8	44
Stage IA	11	5 (31%)	14	2 (12%)	16	5	21
Stage IB	4	4 (50%)	7	1 (12%)	8	1	9
Stage II	9	3 (25%)	9	3 (25%)	12	2	14
Total	24	12 (33%)	30	6 (17%)	36	8	44

*Patients with extrauterine tumor are excluded from the denominator in all percentages.

†Significantly more patients with grade 3 tumors had vascular invasion ($p < 0.05$ by Fisher's exact test).

Table VI. Outcome by site of disease

Site of disease at hysterectomy	*Cases*	*Cancer-related deaths*	
		No.	*%*
A. No residual disease	47	3	6*
B. Residual disease	44	10	23*
Extrauterine	8	3	38
Uterus only	36	7	19
Total	91	13	14
C. Residual disease in uterus only			
Depth of invasion of myometrium			
None	10	0	0
<Half	14	3	21
>Half	12	4	33
Total	36	7	19
Vascular invasion in myometrium			
Present	6	6	100†
Absent	30	1	3†
Total	36	7	19†

*Difference is statistically significant ($p < 0.05$ by chi-square test).

†Difference is statistically significant ($p < 0.01$ by Fisher's exact test).

Forty-seven percent of grade 1 lesions and 41% of grade 2 lesions had residual disease in contrast to 86% of grade 3 lesions. The difference between grade 3 and other lesions was statistically significant at the 5% level ($p < 0.05$ in Fisher's exact test). Correspondingly, 45% of women with glandular or adenoacanthomatous cell types had residual disease compared to 50% of adenosquamous and 100% of anaplastic lesions.

Clinical stage. Table IV shows the disease status by stage and grade in the 91 patients treated by radiotherapy. About 50% of each of Stage Ia, Ib, and II had residual disease after radiotherapy. Within each stage, grade 3 lesions were associated with a higher proportion of residual disease than grade 1 and 2 lesions.

Grade, stage, and extent of disease. Table V shows the relations between grade, clinical stage of disease, and the distribution of residual disease in the pelvis in 44 women (48%) with residual disease after radiotherapy. Eight women had disease outside the uterus. Among 36 women with residual disease confined to the corpus or cervix, there was a trend to more frequent deep invasion with increasing histologic grade. There was also a trend to more frequent vascular invasion in the myometrium with increasing histologic grade.

There was no correlation between stage and either depth of invasion or frequency of vascular involvement.

Reliability of clinical stage. Six of 61 (10%) with clinical Stage I disease had extrauterine disease after radiotherapy. In addition, two with disease confined to the uterus and negative endocervical curettage specimens had disease in the endocervix at hysterectomy. Thus, at least eight patients with clinical Stage I disease (13%) were inaccurately staged. The true proportion would be higher if radiation treatment destroyed tumor in the cervix or pelvis in some patients with preoperative radiotherapy. In the 20 clinical Stage I patients treated by initial operation, three (15%) had extrauterine disease.

Extrauterine disease was present in two of 30 patients with clinical Stage II disease (7%). Again this represents a minimum since pelvic radiation therapy may have destroyed some pelvic metastases.

All 30 clinical Stage II patients had tumor in the endocervical curettings. However, in 13 (43%) the endometrial carcinoma was detached from adjacent benign endocervical tissue with no evidence of invasion of endocervical glands. Only three (10%) had residual disease in the endocervix after radiotherapy. Therefore, the accuracy of assignment of Stage II depends on the accuracy of distinguishing endocervical and endometrial tissue in the fractional curettage.

Follow-up and outcome. Thirteen of 91 patients re-

Table VII. Residual disease and outcome by grade and stage

Grade and stage	No. of cases	Extent of disease				Cancer-related deaths in 2 years	
		Residual disease		Blood vessel and/or pelvic spread			
		No.	%	No.	%	No.	%
Grade 1	62	29	47	6	10	5	8
Grade 2	22	9	41	4	18	5	23
Grade 3	7	6	86	4	57	3	43*
Total	91	44	48	14	15	13	11
Stage IA	43	21	49	7	16	3	7
Stage 1B	18	9	50	2	11	3	17
Stage II	30	14	47	5	17	7	23
Total	91	44	48	14	15	13	11

*Grade 3 differs significantly from Grade 1 ($p < 0.05$ by Fisher's exact test).

Table VIII. Proposed surgical-pathologic staging of endometrial carcinoma

Stage	Criterion
0	Carcinoma in situ
I	The carcinoma is confined to the corpus, including the isthmus, and has involved less than half the depth of the myometrium
II	The carcinoma has involved the endocervix or more than half the depth of the myometrium
III	The carcinoma has involved the blood vessels of the myometrium or has extended to other pelvic structures
IV	The carcinoma has extended outside the true pelvis or has involved the bladder or rectal mucosa

ceiving preoperative radiation and four of 20 patients who underwent initial operation developed recurrences and died of endometrial carcinoma within 2 years of diagnosis. No recurrences or deaths from cancer of the endometrium occurred between 2 and 5 years after diagnosis. One patient died of postoperative complications; six died of other diseases within 5 years, and two died of unknown causes. Six patients were lost to follow-up within 5 years while seven patients with initial operation were not eligible for 5-year follow-up. Complete 5-year follow-up was achieved in 93% of patients with preoperative radiation and in 65% of patients with initial operation.

Outcome by site of residual disease. Table VI shows the frequency of cancer-related deaths in 91 patients who received preoperative radiation, according to the site of residual disease. There were cancer-related deaths within 2 years in 6% of patients without residual disease compared to 23% of women with residual disease. This difference is statistically significant at the 5% level ($p < 0.05$ by Fisher's exact test). Cancer-related deaths were more frequent in patients with extrauterine disease (38%) than in patients with residual disease confined to the uterus (19%).

Thirty-six patients had the disease confined to the uterus. In 12 women, the tumor invaded more than half the depth of the myometrium and four (33%) subsequently died of disease. In 24 women, residual tumor was confined to the endometrium or invaded less than half the depth of the myometrium and three (13%) died of disease. Given these small numbers, the difference in cancer-related deaths according to depth of invasion did not reach statistical significance.

In 30 of 36 patients with residual disease confined to the uterus, there was no invasion of blood vessels and only one cancer-related death (3%). Six women had vascular invasion in the myometrium and all died of cancer of the endometrium. Two of these had blood vessel invasion in the inner half of the myometrium but tumor invaded less than half the depth of the myometrium. In the absence of blood vessel invasion, the presence of residual disease in the myometrium (whether superficial or deep) did not carry a worse prognosis (3% cancer-related deaths) than the absence of residual disease (6% cancer-related deaths).

Eight patients had extrauterine disease and 38% died of cancer. The frequency of cancer-related deaths shows that vascular invasion in the myometrium and extrauterine disease are the worst prognostic features.

In the group of 20 women with initial operation, three (15%) had disease outside the uterus. There were subsequent cancer deaths in 33% of those with extrauterine disease, compared to 18% of those with disease confined to the uterus.

Site of recurrence. All three patients who had no residual disease after radiotherapy and subsequently died of cancer had distant metastases, but no evidence of pelvic recurrence. Among the 10 patients who had residual disease and subsequently died, eight developed recurrence in the pelvis and two developed distant metastases with no evidence of pelvic recurrence. In the initial operation group, three of four patients who developed recurrence and died had distant metastases but no evidence of pelvic recurrence.

Grade or stage correlated with extent of disease and outcome. Table VII correlates the initial characteristics of the tumor—grade and stage—with the outcome, cancer-related deaths, and the intervening variable, extent of disease as measured by (1) residual disease after radiotherapy and (2) presence of blood vessel invasion or extrauterine disease.

As described before, grade 3 lesions were associated with significantly more residual disease than either grade 1 or 2. Moreover, the features with the poorest prognosis (vascular invasion and extrauterine spread) increased in frequency with grade, as did cancer-related deaths.

There were no comparable trends with stage of disease. The slight trend toward more frequent deaths in Stage II was not statistically significant, given the small numbers involved.

In the 20 patients with initial operation, there were cancer-related deaths in 10% of grade 1, 33% of grade 2, and 50% of grade 3 tumors. There were cancer-related deaths in 20% of patients with Stage IA lesions and in 25% of those with Stage IB lesions. These few data tend to support the findings in the group with preoperative radiation, but the numbers are inadequate for firm statistical evaluation.

Comment

This report demonstrates clearly the unreliability of the clinical staging of endometrial carcinoma. At least 10% to 15% of Stage I disease and at least 7% of Stage II disease was understaged. In addition, some Stage II disease might have been overstaged, since in 40% the allocation to Stage II was made on the basis of endocervical curettings in which there was no invasion of the endocervical glands. Since radiotherapy may eradicate disease in the cervix, the accuracy of the endocervical curettage could not be assessed. This unreliability in staging explains, in part, why there was no consistent relation between stage of disease and the extent of disease found at operation or between clinical stage and cancer deaths. Murthy and colleagues[8] reported that about 9% of Stage I disease may be understaged. On the basis of pelvic node involvement, Lewis and colleagues[9] reported that 19% of Stage I disease was understaged, while Morrow and colleagues[10] reported that 11% of Stage I and 37% of Stage II disease may be understaged.

Inconsistency was also found in the FIGO histologic grade, with 20% assigned to different grades in the curettage and hysterectomy specimens. Since this inconsistency occurred to the same extent in those with preoperative radiotherapy and in those with initial operation, there is no evidence that the radiation therapy itself affects the grade of the tumor. However, there was inconsistency in the histologic type in only 2% and none in the anaplastic or adenosquamous lesions. Histologic type is evidently a more reliable characteristic than FIGO grade.

This study demonstrates that in most cases with poor outcomes, features indicating this were present at operation. Anaplastic lesions were associated with more extensive disease and higher mortality rates. The presence of residual disease after radiotherapy significantly increased the frequency of recurrence and death from 6% to 23%. The presence of residual disease in the uterus alone increased the frequency of recurrence and death from 6% to 19%. However, in this series the most significant prognostic features were disease that invaded the blood vessels of the myometrium (deaths in 100%), disease outside the uterus (deaths in 38%), and deep myometrial invasion (deaths in 33%). The latter feature did not reach statistical significance in this series, probably because of the small numbers, but its prognostic significance has been reported in other studies.[4-6] Since vascular invasion increased in frequency with depth of invasion, the former rather than the latter may be the more significant variable. In the absence of these poor prognostic features, the mortality rate was no higher in patients with residual disease than in those without, a finding consistent with a study by the Gynecologic Oncology Group.[9]

These findings suggest the need for adjunctive pelvic radiation and/or chemotherapy for all patients with poor prognostic features at operation, as well as for patients with anaplastic lesions.

The fact that half the patients in this series still had cancer after radiotherapy stresses the limits of this modality in the treatment of endometrial carcinoma. Residual disease was present even in the endometrium and inner myometrium, which should have been most proximal to the intracavitary radiation source. Lewis and co-workers[11] demonstrated that deep myometrial invasion sometimes disappeared with preoperative radiation, with a concomitant loss of prognostic information. In this series there were four patients without poor prognostic features in the irradiated hysterectomy specimen who, nonetheless, died of cancer. This and the unreliability of clinical staging make it evident that appropriate treatment must be based on accurate knowledge of the extent of disease and other prognostic factors in the unirradiated specimen. A further advantage of initial operation is that hysterectomy alone may prove adequate for patients with small, low-grade lesions, such as the two in this series that were completely removed by curettage.

All these factors have led us to favor initial operation followed by radiotherapy in the treatment of early endometrial carcinoma. Initial operation allows the accurate assessment of endocervical involvement, length of the uterine cavity, depth of myometrial invasion, blood vessel invasion, volume of tumor in the corpus, pelvic spread, and pelvic and para-aortic lymph node involvement.

A proposed scheme for pathologic staging of endometrial carcinoma based on findings at initial operation is presented in Table VIII. Based on this scheme, postoperative radiation would be tailored to fit each finding and adjunctive chemotherapy may be initiated earlier.

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Steroid secretion by the postmenopausal ovary

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The concentrations of certain steroids in arterial and ovarian venous blood were measured in 42 postmenopausal women undergoing hysterectomy for uterine disease. A concentration gradient across the ovary was found for testosterone in 21 women, for androstenedione in 16, for estradiol in 15, for estrone in 8, and for dehydroepiandrosterone in 3. On the basis of postulated ovarian blood flow the gradient would result in a major contribution to the blood production rate for testosterone in about 50% of women and for estradiol in less than 20%. For androstenedione, dehydroepiandrosterone, and estrone the gradients did not signify important ovarian secretion. There was no evidence for secretion of dihydrotestosterone, estrone sulfate, or dehydroepiandrosterone sulfate. There were no apparent clinical correlates to indicate in which women the ovary was secreting steroids. (AM. J. OBSTET. GYNECOL. 138:564, 1980.)

IN PREMENOPAUSAL WOMEN, circulating estradiol and estrone arise almost entirely as a result of ovarian secretion,[1-3] although some estrone enters the blood as a result of peripheral conversion of androstenedione.[1, 3] Measurements of ovarian vein steroid levels in small groups of postmenopausal women have indicated that the postmenopausal ovary does not secrete estrogens[4] but rather androgens. The small amounts of estrogen that persist in peripheral blood have been postulated to arise entirely from the peripheral conversion of the ovarian and adrenal androgens.[3, 5] However, in recent measurements of estrogen blood production rates in postmenopausal women, we found a number of women in whom peripheral androgen conversion could not explain the entire amount of estrogen.* Others[6] also have noted the same discrepancy between estrogen production rates and the androgen contribution to these rates.

We felt it possible that in some women ovarian estrogen secretion might continue after the menopause. Therefore, we measured arterial vein and ovarian vein blood levels of several steroids in postmenopausal women. Our findings are not in full agreement with previous studies but suggest a spectrum of ovarian function in postmenopausal women.

Material and methods

All subjects were women who had had no regular menstrual cycles for at least 14 months prior to operation and were undergoing operation for uterine disease, usually endometrial carcinoma. The mean age (±SE) of the women was 62 ± 1 year, the mean number of years after menopause was 12 ± 1, and the mean body weight was 153 ± 7 pounds. Fourteen of the women had received estrogen therapy prior to operation but none in the month before operation. At the time of operation 2 to 5 ml blood samples were collected from a radial or ovarian artery and from veins draining both ovaries. The blood samples were centrifuged, and the plasma was harvested and stored frozen until analyzed. Samples were analyzed by radioimmunoassay for estrone,[7] estradiol,[7] testosterone,[7] 5α-dihydrotestosterone,[7] and androstenedione[7] in 42 subjects and for estrone sulfate, dehydroepiandrosterone sulfate, and dehydroepiandrosterone[8] in 10 of these subjects.

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Supported by Grant No. AG-00058 from the National Institute on Aging.

Received for publication February 19, 1980.

Revised June 24, 1980.

Accepted June 30, 1980.

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**Longcope, C.: Unpublished data.*

0002-9378/80/210564+05$00.50/0 © 1980 The C. V. Mosby Co.

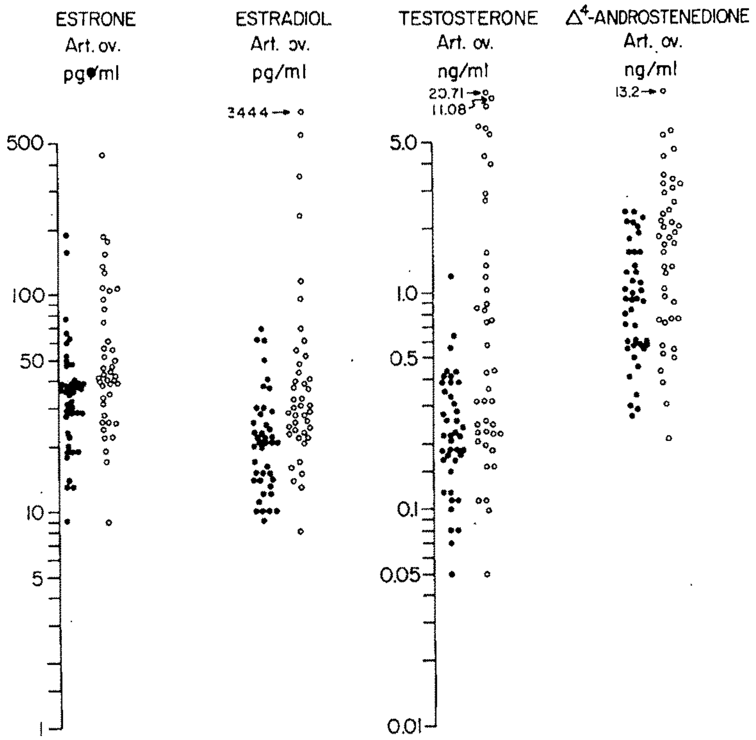

Fig. 1. Concentrations of estrone (picograms per milliliter), estradiol (picograms per milliliter), testosterone (nanograms per milliliter), and Δ^4-androstenedione (nanograms per milliliter) in arterial (*Art.*, •) and ovarian (*ov.*, o) vein blood of all subjects.

All samples from one subject were always measured in the same assay.

Results

The assays used in these studies have intra-assay coefficients of variation of <12%. We considered the venous-arterial difference was significant if it exceeded the sum of each concentration multiplied by two times the maximal coefficient of variation, i.e., a gradient was considered as significant if the following were satisfied: $V\text{-}A > 0.24A + 0.24V$, where A = arterial concentration and V = ovarian vein concentration.

Steroid concentrations in arterial and ovarian plasma are shown in Figs. 1 and 2.

Of the 42 samples examined, gradients were found in eight for estrone, in 15 for estradiol, in 16 for Δ^4-androstenedione, in 21 for testosterone, and in three for dehydroepiandrosterone. We were not able to demonstrate a gradient for dihydrotestosterone, estrone sulfate, or dehydroepiandrosterone sulfate in any of the samples measured although gradients for one or more of the previous steroids were noted in seven of these.

Of the 42 women studied, 27 (Table I) had a significant gradient for one or more steroids. Only two women had a gradient for estrogen and not androgen and in both cases the gradient was for estradiol. Eleven subjects showed a gradient for androgen and not estrogen. The gradient was for testosterone only in five of these, for Δ^4-androstenedione only in two, and for both in four. Gradients were found for these four major steroids in only four subjects. When analyzed by the chi-square test, previous hormone therapy was not a significant factor as to the presence or absence of a gradient.

In 20 of the 27 subjects the gradient was bilateral and in the other seven a gradient could be detected across only one ovary.

The overall mean (± SE) arterial concentration for estrone was 39 ± 4 pg/ml. In the eight subjects in whom a gradient was found, the mean concentrations in the arterial and ovarian venous plasma samples were 30 ± 6 and 162 ± 44 pg/ml, respectively.

For estradiol, the overall mean arterial concentration was 24 ± 2 pg/ml. In the 15 subjects in whom a gradient was found the mean concentrations in the arterial and ovarian venous plasma samples were 24 ± 4 and 344 ± 244 pg/ml, respectively.

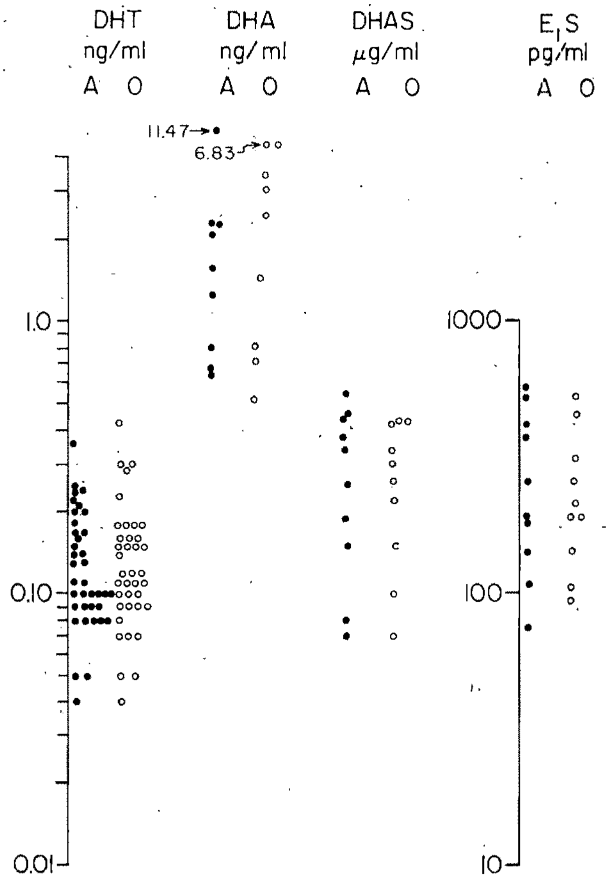

Fig. 2. Concentrations of dihydrotestosterone (*DHT*, nanograms per milliliter), dehydroepiandrosterone (*DHA*, nanograms per milliliter), dehydroepiandrosterone sulfate (*DHAS*, micrograms per milliliter), and estrone sulfate (E_1S, picograms per milliliter) in arterial (*A*, ●) and ovarian (*O*, ○) vein blood.

The overall mean concentration of testosterone in arterial plasma was 0.27 ± 0.03 ng/ml. In the 21 subjects in whom a gradient could be demonstrated the mean values in arterial and ovarian venous blood samples were 0.32 ± 0.05 and 3.53 ± 1.05 ng/ml, respectively.

For Δ^4-androstenedione and overall mean concentration in arterial plasma was 1.07 ± 0.10 ng/ml. In the 16 subjects in whom a gradient was found the mean concentrations in arterial and ovarian plasma were 1.45 ± 0.26 and 3.52 ± 0.76 ng/ml.

In three subjects a gradient was noted for dehydroepiandrosterone. In these subjects the arterial concentrations were 0.8, 2.1, and 2.3 ng/ml and the ovarian vein concentrations were 3.1, 3.6 and 4.6 ng/ml, respectively. All these subjects also had gradients for testosterone and/or androstenedione.

For dihydrotestosterone, estrone sulfate, and dehydroepiandrosterone sulfate (Fig. 2) no significant gradients could be found. The mean concentrations in arterial and ovarian plasma for each steroid were 0.14 ± 0.01 and 0.14 ± 0.01 ng/ml, 284 ± 55 and 249 ± 45 pg/ml, and 0.28 ± 0.05 and 0.26 ± 0.04 μg/ml, respectively.

Comment

According to our data some but not all postmenopausal ovaries secrete estrogens and androgens. In our series a gradient in steroid concentration across the ovary was noted in 65% of the subjects. However, only a few (10%) of the patients showed a gradient for all four major steroids. Whether the conditions under which the blood samples were obtained could play a role in this is uncertain. Stress has been noted to decrease the testicular secretion of steroids,[7] so it is possible our data slightly underestimated the degree of the gradient and perhaps the number of subjects who would show a gradient. Under somewhat similar operative conditions, a significant gradient was found in 100% of a group of premenopausal subjects,[9] so it is unlikely that our results showing the presence or absence of gradients are influenced to a major degree by the operative conditions.

It should be noted, however, that the mean values for estrone, estradiol, testosterone, and androstenedione were all somewhat, but not significantly, higher than values found in a large group of postmenopausal women. This is probably secondary to the stress of operation, which causes an increase in androstenedione secretion from the adrenal and a secondary rise in the other steroids as a result of peripheral conversion.[7] The majority of our subjects had endometrial carcinoma. However, there appears to be no significant difference in circulating levels[10] or production rates[11] of androgens and estrogens between postmenopausal women with endometrial carcinoma and normal postmenopausal women of similar weights. Therefore, it is probable that our findings would not be influenced by the uterine disease per se.

The mean weight of our patients was 153 ± 7 pounds and the mean height was 64 ± 1 inch. Therefore, they were at least 20% overweight, and while adipose tissue can influence the aromatization of androgens,[12] its effect on ovarian secretion is not clear. However, the mean weight of our patients with a gradient (150 ± 7 pounds) was not different ($p > 0.1$) from the mean weight of our patients without a gradient (159 ± 15 pounds). Therefore, it would appear that in our series the presence or absence of a gradient was not influenced to any major degree by the degree of adiposity.

Steroids are associated with red cells,[1] and Holzbauer and Vogt[13] noted that the venous-arterial gradient across the adrenal for aldosterone was greater in whole

Table I. Steroid concentrations in women in whom a concentration gradient was found across the ovary

Subject No.	*Testosterone (ng/ml)*		*Androstenedione (ng/ml)*		*Estradiol (pg/ml)*		*Estrone (pg/ml)*	
	Arterial	*Ovarian*	*Arterial*	*Ovarian*	*Arterial*	*Ovarian*	*Arterial*	*Ovarian*
1	0.24	11.08	0.27	2.66	10	38	14	26
2	0.19	5.54	NS*		22	549	19	446
3	0.31	5.32	1.11	3.43	24	41	NS	
4	0.22	4.00	0.92	3.24	NS		NS	
5	0.05	0.25	—	—	NS		NS	
6	0.28	1.37	0.57	4.74	NS		NS	
7	0.22	2.77	NS		NS		NS	
8	0.20	0.59	NS		NS		NS	
9	0.35	0.86	NS		NS		NS	
10	0.19	2.93	0.81	3.22	50	99	38	135
11	0.44	0.90	0.41	1.81	20	44	39	105
12	0.17	0.76	—	—	29	353	66	185
13	0.55	1.56	2.40	5.55	62	119	NS	
14	0.42	1.18	1.52	2.42	30	52	NS	
15	0.23	1.05	NS		28	62	NS	
16	0.43	7.32	1.80	13.23	10	24	NS	
17	0.21	0.43	NS		NS		NS	
18	1.20	20.71	1.26	5.68	26	3444	30	177
19	0.33	4.44	NS		13	40	NS	
20	0.11	0.23	0.94	3.14	NS		NS	
21	0.39	0.85	1.05	4.42	NS		NS	
22	NS		0.58	2.23	12	231	13	129
23	NS		NS		11	23	NS	
24	NS		NS		NS		22	95
25	NS		0.73	2.96	16	48	NS	
26	NS		0.56	1.33	NS		NS	
27	NS		1.58	3.53	NS		NS	

*NS = Arterial and ovarian concentrations were not significantly different, i.e., O-A < 0.24O + 0.24A.

blood than in plasma. However, in several subjects we measured both blood and plasma concentrations and no differences in the presence or amount of the gradient were found. We feel, therefore, that the use of plasma concentrations gives meaningful data as to gradients and their importance.

It has been postulated that there is a diminution in ovarian function as the time from menopause increases. If this were so, then we would have expected to find that women with gradients were younger than those without. However, the mean age of the subjects with any type of gradient was 63 ± 1 year, which is not significantly different from those without such a gradient, whose mean age was 61 ± 2 years. Therefore, there is no apparent relationship between the age of the subject and the presence of a gradient.

We found, as others have found,[4] that more postmenopausal ovaries show a gradient for androgens, especially testosterone, than for estrogens. This is probably related in part to the lack of follicles in postmenopausal ovaries, since the stroma is capable of synthesizing androgens, but the bulk of estrogen synthesis appears to take place in the granulosa.[14] Most postmenopausal ovaries probably contain few functioning follicles.[15]

Compared to the mean gradients noted in the premenopausal women (1,089 and 10,405 pg/ml and 79.85 ng/ml for estrone, estradiol, and androstenedione, respectively[9]), the gradients noted in our group (132 pg/ml and 320 pg/ml and 2.9 ng/ml) were considerably lower, but the gradients for testosterone (3.5 and 3.2 ng/ml) were similar for the premenopausal and postmenopausal groups, respectively. It would appear, therefore, that in postmenopausal women ovarian testosterone secretion is more often maintained but the secretion of estrone, estradiol, and androstenedione decreases to a considerable degree.

The presence of a concentration gradient across the ovary is necessary to show that the ovary is secreting steroids. However, in order to calculate the amount of steroid secreted, one must know the blood flow of the ovary, and there are few data available for this. Baird and Fraser[2] calculated an ovarian plasma flow of 60 L/day in premenopausal women, but it is not clear whether the ovarian plasma flow in postmenopausal women would be similar to that. However, using that figure and the mean value for the A-V difference in women with a gradient, we calculated maximal ovarian secretion rates of 8, 19, and 180 μg/day and 0.1 ng/day for estrone, estradiol, testosterone, and androstenedione, respectively. We do not know the production rates of steroids in these particular women, but using mean values reported previously[3] we calculated that even when a gradient exists for estrone, androstenedione, and dehydroepiandrosterone across the ovary the ovary is not an important source of these steroids. However, it would appear that the gradients noted for estradiol and testosterone reflect ovarian secretion which could be important to the overall production rate of these steroids. As noted, however, ovarian estradiol production would be important in less than 20% of our group whereas close to 50% of the women would have ovaries secreting major amounts of testosterone.

Our conclusions are somewhat at variance with those noted by Judd and associates,[4] who found only a small gradient for estradiol in two of five subjects and thus concluded that the postmenopausal ovary is not capable of major estradiol secretion. We believe that the explanation for our different conclusions lies in the larger number of patients studied. Vermeulen[16] also concluded that the postmenopausal ovary was not an important source of estrogens, but he used indirect means and did not measure ovarian vein steroid concentrations.

We found no evidence in our subjects for ovarian secretion of estrone sulfate, dihydrotestosterone, or dehydroepiandrosterone sulfate, which is not unexpected since the premenopausal ovary does not secrete important amounts of these steroids.

We were not able to differentiate the secretory from the nonsecretory ovary on clinical grounds. Therefore, the ovary must be considered a potential source of estradiol and testosterone in all postmenopausal women.

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Presence of gap junctions in the myometrium of women during various stages of menstruation

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Myometrial tissues from 36 women who underwent hysterectomy at various stages of the menstrual cycle were examined in the electron microscope for the presence of gap junctions. Gap junctions were present between smooth muscle cells in some of the tissues from women with dysmenorrhea, during menstruation and at other times. The suggestion is made that gap junctions may form between myometrial cells in nonpregnant women in response to physiologic or pathologic stimuli, such as the production of prostaglandins, and the presence of the contacts may result in contractions of the uterus. (AM. J. OBSTET. GYNECOL. 138:569, 1980.)

GAP JUNCTIONS are present in increased frequency between smooth muscle cells of the myometrium of rats, sheep, guinea pigs, and human beings during labor and parturition.[3-7] These specialized cell-to-cell contacts are thought to lower the impedance to the flow of current between cells, and their presence to result in coordinated and synchronized muscle activity.[3-7] Changes in the levels of steroid hormones and prostaglandins are thought to initiate the formation of gap junctions.[3-7]

Menstruation and ovulation are known to be preceded and controlled by changes in estrogens, progesterone, and prostaglandins.[1, 9-12] Menstruation is frequently accompanied by painful contractions of the muscles of the uterus (dysmenorrhea). These contractions can be alleviated by treatment with drugs that inhibit the synthesis of prostaglandins.[2, 13-15]

The purpose of this study was to quantitatively examine myometrial tissues from nonpregnant women who underwent hysterectomy at various times during the menstrual cycle. Gap junctions were found in some women during menstruation and at other times in the cycle. The presence of the junctions may be related to menstruation, ovulation, and various pathologic or gynecologic conditions.

Material and methods

Small segments (2 cm by 2 mm by 5 mm) of myometrial tissues from the fundus of the uterus were obtained from women who underwent hysterectomy for a variety of medical reasons. The tissues were fixed in glutaraldehyde (2% glutaraldehyde + 4.5% sucrose 1.5 mM $CaCl_2$ in 75 mM sodium cacodylate buffer, pH 7.4) for 2 to 24 hours. After the initial fixation, longitudinal strips were cut from the outer portion of the original tissues, washed in buffer for 60 minutes, and postfixed in 2% osmium tetroxide (in cacodylate buffer 50 mM, pH 7.4) for 90 minutes. All tissues were stained en bloc with saturated uranyl acetate solution for 30 minutes. The tissues were dehydrated in graded ethanols and embedded in Spurr resin. The tissues were oriented in molds so that the muscle cells could be cut in transverse orientation. Sections of the muscle were cut on a Porter-Blum (MT2-B) ultramicrotome and mounted on 200 mesh grids and stained with lead citrate.

All tissues were examined in a Philips electron microscope (Model 301). To quantitate the presence of gap junctions, 20 to 22 nonoverlapping photographs from each tissue were taken of one section overlying several

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Supported by grants from the Medical Research Council of Canada and the World Health Organization.

Received for publication May 30, 1980.

Revised July 8, 1980.

Accepted July 14, 1980.

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Table I. Presence of gap junctions (GJs) in nonpregnant women during various stages of menstrual cycle*

	Days from start of LNMP	*No. of women*	*No. of women with GJs*	*No. of GJs*	*Length of membrane surveyed (μm)*
Menses	1-2	3	1	7	2,846
	3-4	0	0	0	—
	5-6	3	1	1	2,055
	7	1	1	8	785
	8-9	2	0	0	2,179
	10-11	0	0	0	—
	12-13	2	0	0	2,007
Ovulation	14	2	2	7	1,826
	15-16	1	0	0	753
	17-18	0	0	0	—
	19-20	1	0	0	920
	21-22	0	0	0	—
	23-24	1	1	2	1,298
	25-26	2	1	4	1,746
	27-28	2	0	0	1,576
	29-30	2	1	1	1,917
	31-32	0	0	0	—
	33-34	1	1	10	832
	35-36	4	2	10	3,379
	37-38	1	0	0	1,055
	39-40	1	0	0	871
	>41	4	0	0	3,786
	Irregular	3	1	5	3,111
	Total	36	12	55	

*The number of gap junctions (No. of GJs) found in a series of electron micrographs taken of each tissue (20 to 22 photographs) from 36 women who underwent hysterectomy at various times after start of last normal menstrual period (LNMP). Length of membrane surveyed refers to total length of smooth muscle cell membrane measured in photographs for women at each time.

grid squares. Lengths of smooth muscle cell membrane surveyed for each tissue were determined from the series of photographs printed at a final magnification of ×33,000. The smooth muscle cell membrane length was estimated as described previously[4] by superimposing a transparent grid over each photograph. Gap junctions were identified as membrane structures with either five lines or seven lines in photographs or negatives enlarged to ×100,000 magnification.

All relevant information on each patient, as to age, medications used, obstetric and gynecologic history, including menstrual status, was obtained and recorded on clinical data forms. This project was approved by the Ethics Committee of the respective institutions concerned.

Results

Tissues from 36 women in various stages of the menstrual cycle were examined in this study (Table I). There were tissues from 12 women in which at least one gap junction was found between muscle cells in the series of photographs.

There were seven women who were menstruating when the tissues were collected (days 1 to 7 of the menstrual cycle, Table I). Gap junctions were found in tissues from three of these women. The junctions were present in tissues from two of three women taken at the predicted time of ovulation (days 14 to 16 of the cycle). Gap junctions were also found in tissues from some women who had irregular menstrual periods and whose menstrual cycles were extended.

Table II gives a brief description of four women from whom tissues were obtained and found to contain a high frequency of gap junctions (range from 6 to 12 gap junctions per 1,000 μm of membrane surveyed). Two of the women (Nos. 504 and 482) were menstruating, and two (Nos. 449 and 327) reported irregular periods. Three of the women (Nos. 504, 327, and 449) suffered from severe to moderate dysmenorrhea. No dysmenorrhea was noticed by one woman (No. 482) who had started menstruating 1 day before the tissue was obtained.

Figs. 1 through 4 are electron micrographs of myometrial tissues from two of the women in this study. Fig. 1 is a low-magnification micrograph of many smooth muscle cells in transverse section. Two

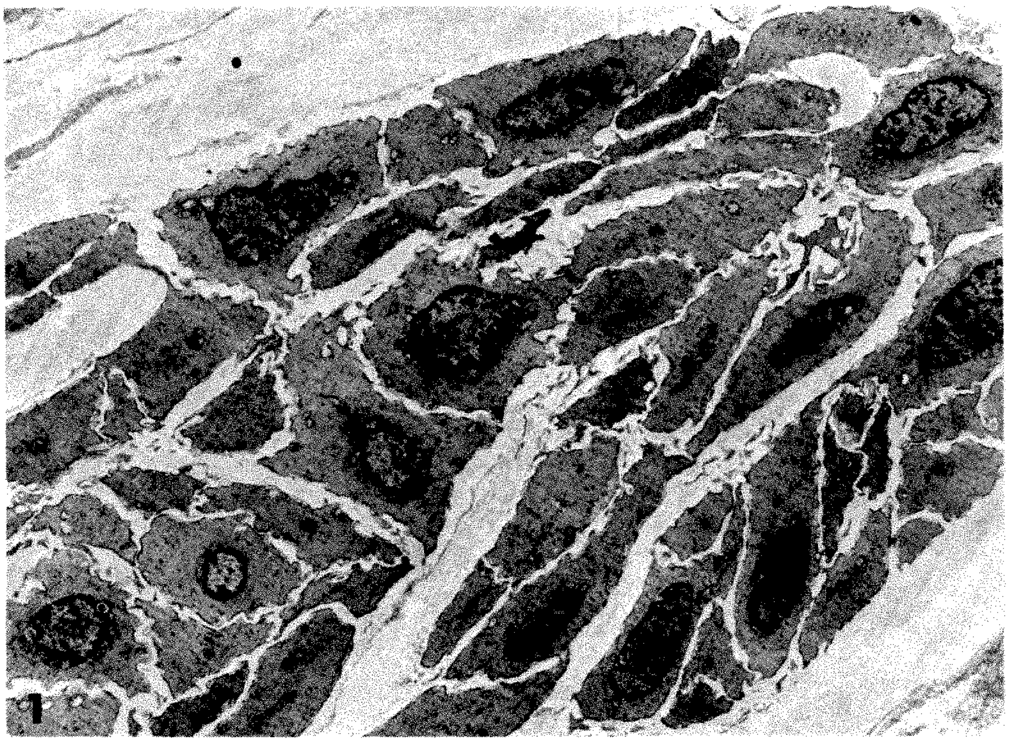

Fig. 1. Patient (No. 059), 42 years old, with irregular menstrual periods, dysmenorrhea, and pathologic findings of severe dysplasia. Low-magnification electron micrograph showing profiles of uterine smooth muscle cells in transverse orientation. (×3,400.)

gap junctions between smooth muscle cells from patient No. 504 (Table II) are shown in Fig. 2. At high magnification, the seven-lined appearance of the gap junction is evident (Fig. 3). The organization of contractile filaments within a smooth muscle cell is shown in Fig. 4.

Comment

Our findings show conclusively that gap junctions are present between smooth muscle cells of the uterus in nonpregnant women, but do not show unequivocally that the junctions are present at any particular time during the menstrual cycle. Although the junctions were present in some women during menstruation, at predicted times of ovulation, and at other times (Table I), these findings may be fortuitous rather than related to any physiologic changes occurring during these times.

In most of the tissues, gap junctions were present in very low frequency. The fact that no gap junctions were found in tissues from 24 women (Table I) may reflect a scarcity rather than a total absence. That gap junctions were present in high frequency in tissues from four women (Table II) may be related to the

Table II. Women with high frequency of gap junctions (GJs) between smooth muscle cells of myometrium*

Patient No.	
504	Age 39 years Found 8 GJs in 785 μm membrane Start LNMP 7 days Severe dysmenorrhea Large myomatous uterus
327	Age 50 years Found 10 GJs in 832 μm membrane Start LNMP 34 days; irregular menstruation Severe dysmenorrhea; bed rest Endometriosis posterior aspect of uterus
449	Age 27 years Found 7 GJs in 1,032 μm membrane Start LNMP 36 days; irregular menstruation Moderate to severe dysmenorrhea Severe dysplasia
482	Age 36 years Found 7 GJs in 1,157 μm membrane Start LNMP 1 day No dysmenorrhea No pathologic condition

*Brief description of four women from whom tissues were obtained and found to contain many gap junctions between myometrial smooth muscle cells. Shown are age of patients, number of gap junctions (GJs) found in length of membrane surveyed, time from start of last normal menstrual period (LNMP), history of dysmenorrhea, and pathologic condition.

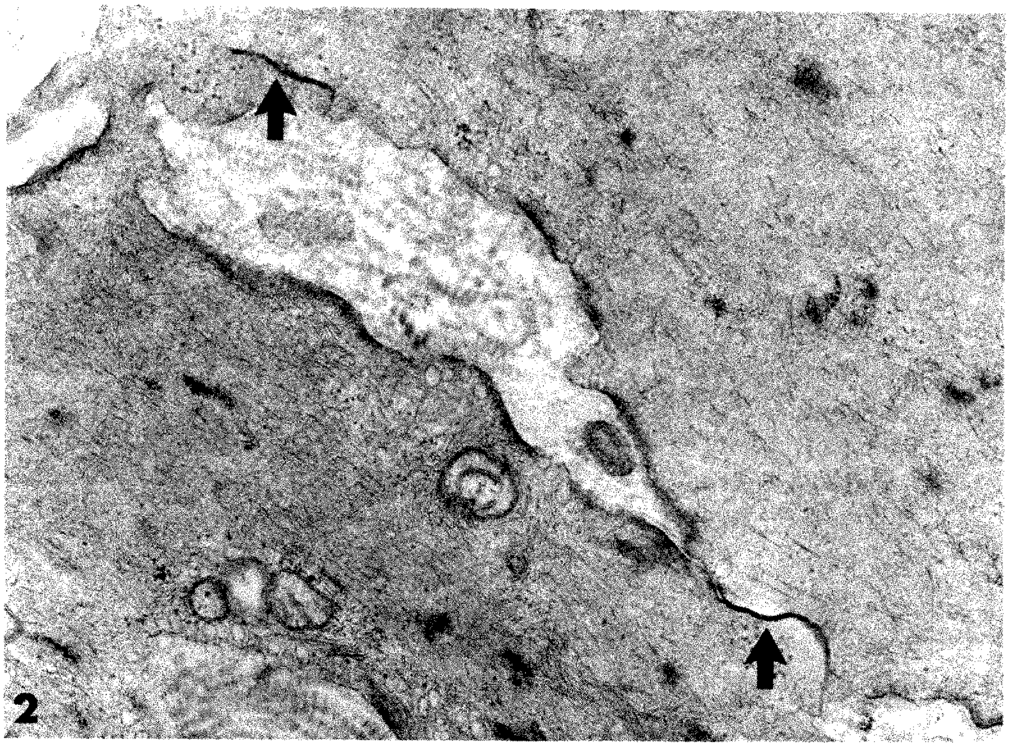

Fig. 2. Patient No. 504 (see Table II). Intermediate-magnification electron micrograph of portions of two myometrial smooth muscle cells with two gap junctions *(arrows)* present between the cells. (×34,000.)

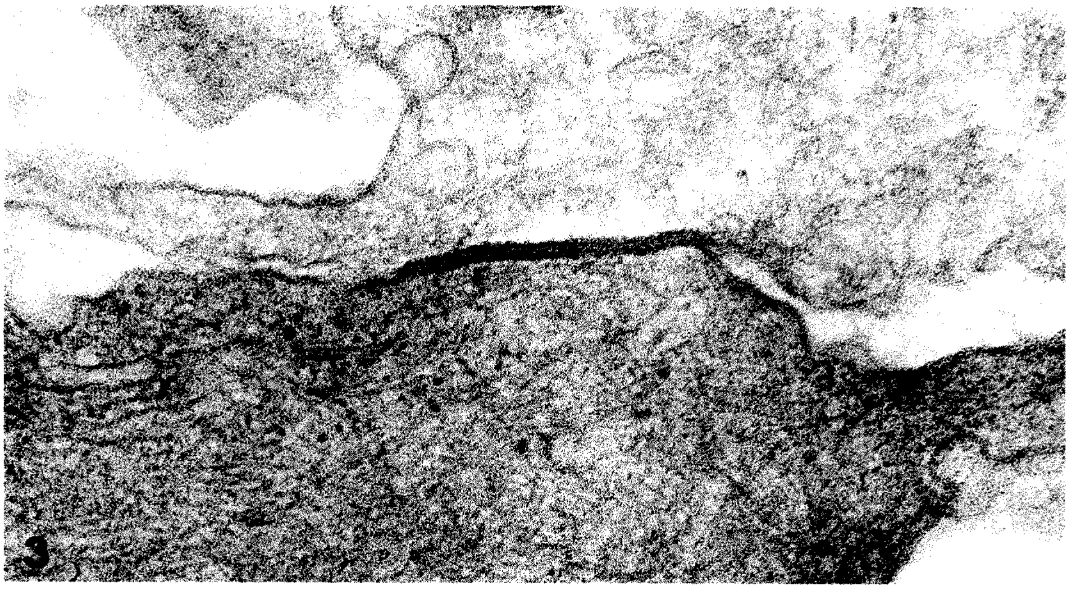

Fig. 3. Patient No. 504. Electron micrograph of gap junction at high magnification showing seven-lined appearance of the contacts. (× 105,000.)

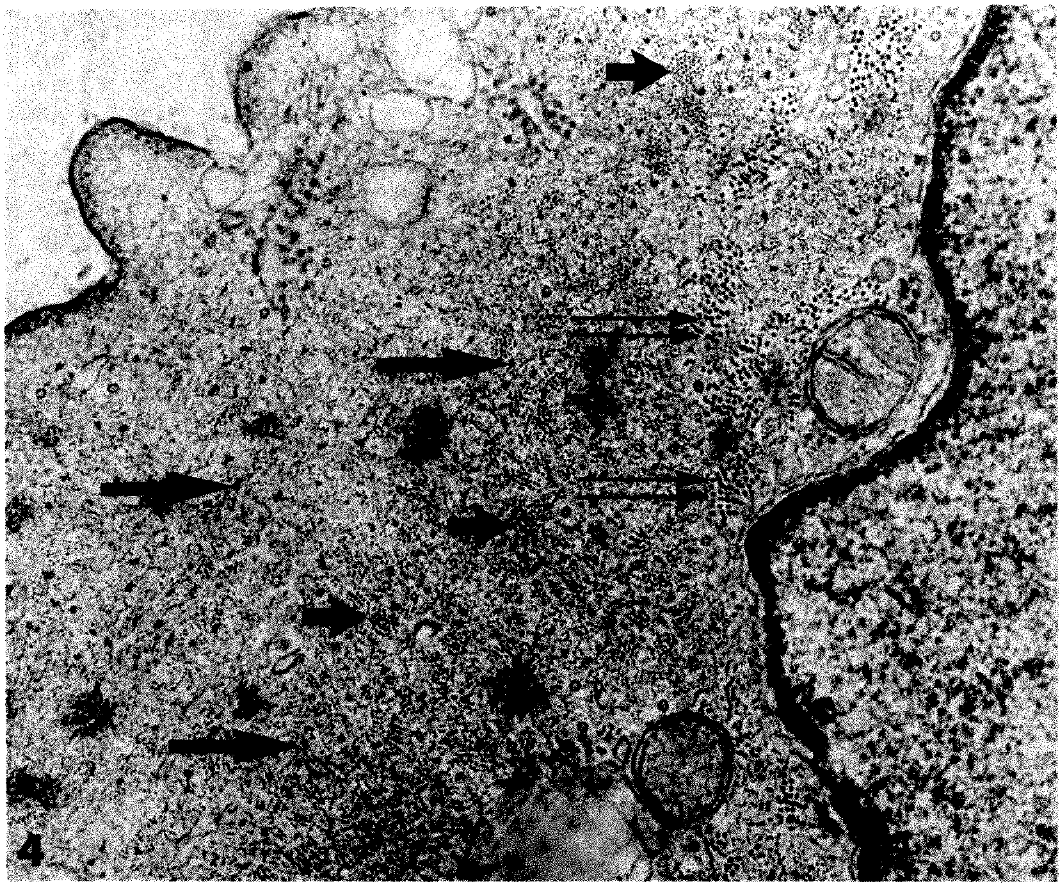

Fig. 4. Same patient as in Fig. 1. Portion of uterine smooth muscle cell at high magnification showing actin *(small arrows)*, myosin *(large arrows)*, and intermediate *(double arrows)* filaments. (×60,000.)

probability of finding the junctions in any tissues where they exist in low frequency.

Gap junctions are thought to be areas of intercellular communication between cells.[8] In the myometrium, these cell-to-cell contacts are present in high frequency in pregnant animals just prior to, during, and immediately after parturition.[3-7] The junctions are thought to synchronize and coordinate the muscle activity of labor.[4] In experimental animals and human beings, the changes in estrogen, progesterone, and prostaglandins which precede labor are thought to initiate the formation of gap junctions.[3-7] Estrogen stimulates the formation of gap junctions, whereas progesterone and prostaglandin inhibitors prevent the formation of gap junctions.[4, 6, 7] The findings of the present study do not necessarily support the above-mentioned mechanism for control of the formation of gap junctions.

The changes in the synthesis of steroid hormones[9, 11, 12] and prostaglandins[1, 10] which accompany menstruation and ovulation may stimulate the formation of gap junctions and lead to muscle activation and dysmenorrhea. Concentrations of prostaglandins in the uterus are elevated in women with dysmenorrhea.[2, 13] Agents which inhibit the synthesis of prostaglandins have recently been shown to give relief from the pain of dysmenorrhea.[2, 13-16] These agents may prevent the formation of gap junctions between myometrial cells in association with changes in the levels of prostaglandins.

Gap junctions were found in some of the tissues in this study from menstruating women (Table I), and in some cases the junctions were associated with dysmenorrhea (Table II). The presence of gap junctions at other times may be related to various pathologic conditions or gynecologic problems from which these women suffer. All of the tissues examined in this study were from women who underwent hysterectomy for some problem related to the uterus. Thus, the presence of gap junctions in these tissues may not exactly correspond to what occurs normally under physiologic conditions. It should be noted that gap junctions are not normally present in myometrial tissues from nonpregnant animals, but they do develop when the same tissues are incubated in vitro.[4-7]

The findings of this study suggest a possible anatomic basis for activation of uterine smooth muscle during the menstrual cycle and an approach to control of these events. Further studies are needed to define the role of gap junctions in the myometrium as related to dysmenorrhea and other gynecologic or pathologic conditions.

We thank Jeanette Zeagler and Debbie Merrett for their expert technical assistance, and Connie Leija, LVN, for her help in obtaining the specimens and clinical data.

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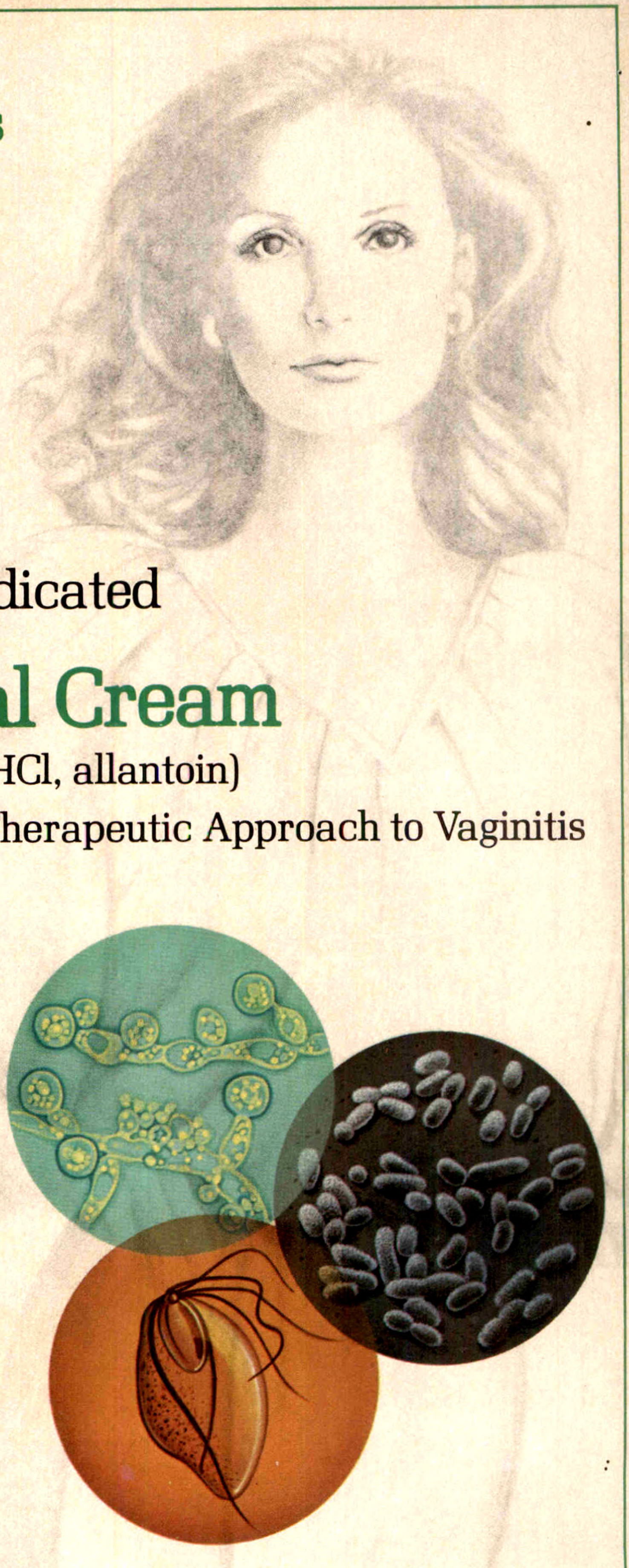

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[1] Federal Register, Vol. 37, 15028 (July 27, 1972)

[2] Fleury, F., Gardner, K., Medical Times, December, 1978

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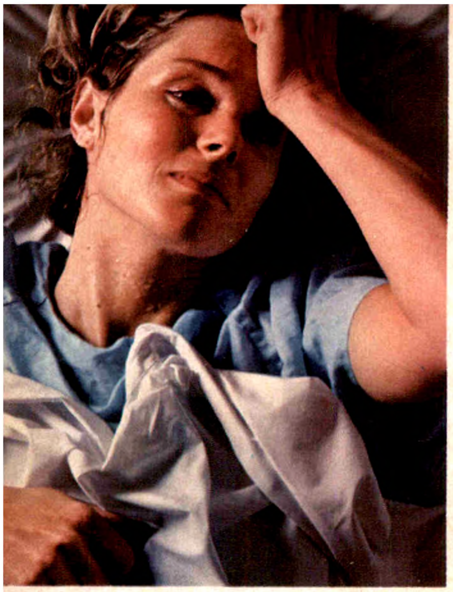

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Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year.[1-3] This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.[4] The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment[1] and on estrogen dose.[3] In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration;[3] it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare.[5,6] This risk has been estimated as not greater than 4 per 1000 exposures.[7] Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis,[8-12] epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects.[13-16] One case control study[16] estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (Conjugated Estrogens Tablets, U.S.P.) for oral administration contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters.

INDICATIONS: Based on a review of PREMARIN Tablets by the National Academy of Sciences—National Research Council and/or other information, FDA has classified the indications for use as follows:

Effective:

1. Moderate to severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms, and they should not be used to treat such conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.
7. Breast cancer (for palliation only) in appropriately selected women and men with metastatic disease.
8. Prostatic carcinoma—palliative therapy of advanced disease.
9. Postpartum breast engorgement—Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated that the incidence of significant painful engorgement in patients not receiving such hormonal therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for this indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with the use of large doses of estrogens.[19]

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

"Probably" effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast,[17] although a recent study has raised this possibility.[18] There is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.[17]

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement.[19-22] Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction.[23-30] Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users.[31,32] An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives.[33,34] If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown[35] to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives.[36] Increased blood pressure may occur with use of estrogens in the menopause[37] and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Oral contraceptives appear to be associated with an increased incidence of mental depression.[23a] Patients with a history of depression should be carefully observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not complete.

The following changes may be expected with larger doses of estrogen

a. Increased sulfobromophthalein retention.
b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.
d. Impaired glucose tolerance.
e. Decreased pregnanediol excretion.
f. Reduced response to metyrapone test.
g. Reduced serum folate concentration.
h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome, amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair, hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION: 1. *Given cyclically for short term use only:* For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (*e.g.*, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

2. *Given cyclically:* Female hypogonadism. Female castration. Primary ovarian failure. Osteoporosis.

Female hypogonadism—2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days' duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 7.5 mg daily in divided doses, for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day of bleeding.

Female castration and primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis (to retard progression)—1.25 mg daily, cyclically.

3. *Given for a few days:* Prevention of postpartum breast engorgement—3.75 mg every four hours for five doses, or 1.25 mg every four hours for five days.

4. *Given chronically:* Inoperable progressing prostatic cancer—1.25 to 2.5 mg three times daily.

Inoperable progressing breast cancer in appropriately selected men and postmenopausal women—10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED: PREMARIN (Conjugated Estrogens Tablets, U.S.P.) No. 865—Each *purple* tablet contains 2.5 mg in bottles of 100 and 1,000. No. 866—Each *yellow* tablet contains 1.25 mg in bottles of 100 and 1,000. Also in unit dose package of 100. No. 867—Each *red* tablet contains 0.625 mg in bottles of 100 and 1,000. Also in unit dose package of 100. No. 868—Each *green* tablet contains 0.3 mg in bottles of 100 and 1,000.

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CURRENT DEVELOPMENTS

Current concepts of amniotic fluid dynamics

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Amniotic fluid at term in the human is the product of numerous exchanges with the fetal and, indirectly, the maternal compartments. Our current information on the formation, circulation, and removal of this fluid is limited but provides an interesting concept of the steady-state regulation of this space. Further confirmation of this model comes from data describing drug exchange between mother, fetus, and amniotic fluid. (AM. J. OBSTET. GYNECOL. 138:575, 1980.)

THE REGULATION of amniotic fluid volume and composition remains a fascinating but modestly understood subject. Increased use of this fluid space for evaluation of fetal welfare by means of individual solute measurement (i.e., phospholipids, creatinine, Δ optical density at 450 nm, and various enzymes) and other techniques (i.e., ultrasound, amniography) has intensified our interest in the formation, circulation, and removal of amniotic fluid. There is now general appreciation that alterations of this fluid compartment in many instances parallel changes in the fetus, indicating that the exchanges between fetus and amniotic fluid are, therefore, sizable and consistent and result in a relatively short lag time before amniotic fluid evidence of fetal status appears. However, we have only a rudimentary understanding of the basic physicochemical mechanisms and pathways involved in the regulation of amniotic fluid volume and composition. It is the purpose of this discussion to review and evaluate current knowledge in this important area.

Obsolete concepts

A number of hypotheses advanced in past years to explain amniotic fluid formation and regulation can be reasonably discarded or revised for outright lack of evidence or misinterpretation of data presented. It is appropriate to begin our consideration of this complex subject by critically reviewing several physiologic concepts that, although clearly outdated and no longer supportable in light of current evidence, are still often mentioned in discussions of amniotic fluid physiology.

Secretion of water or amniotic fluid by amniotic epithelium or chorion laeve.[1-3] These membranes have been thoroughly examined over the last two trimesters of pregnancy, as both isolated in vitro preparations and in vivo.[4-8] There is no evidence for the active transport of water across these tissue layers under any circumstances. Water crosses these membranes only in response to a chemical potential gradient, osmotic or hydrostatic, and in the absence of such a gradient no net movement of water occurs. Thus, the "secretion" of fluid or water by the tissue layers surrounding the amniotic cavity simply does not occur. If a net movement of water should take place across amnion or chorion laeve in the third trimester, it would occur by a passive process or by osmosis from the amniotic sac to the maternal compartment, mediated by the chemical potential gradient between hypotonic amniotic fluid and isotonic maternal fluids. Only a very small net transfer of water could occur by this pathway, however, since the vascularity of maternal tissue in proximity to chorion laeve and amnion is sparse.

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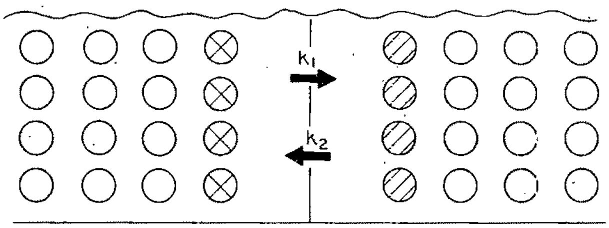

Fig. 1. Algebraic sum of unidirectional fluxes ($k_1 - k_2$) gives the net flux or net transfer of isotopic water from which net transfer of solvent water can be calculated. However, in the case of dilute solutions (i.e., most biologic fluids) the random movement of water molecules in one direction (unidirectional flux) across a permeable tissue layer will be enormous compared to the net flux, thus making accurate calculation of a net transfer of water almost impossible. k_1 = Unidirectional flux or diffusion of ⊗-labeled water molecules across permeable membrane from Side 1 to Side 2; k_2 = unidirectional flux or diffusion of ⊘-labeled water molecules from Side 2 to Side 1; $k_1 - k_2$ = net flux of isotopically labeled water molecules from which the net transfer of solvent water would be calculated. Open circles represent unlabeled water molecules.

Transfer of water between amniotic fluid and fetal vessels in the umbilical cord.[9, 10] Again, water can only cross the exchange surface between these large vessels and the amniotic cavity in response to chemical potential gradients. Although the isotonic fetal plasma can be expected to reabsorb some water from the hypotonic amniotic fluid, in fact, the very small ratio of the vessel wall surface area to the flow provided by large blood vessels makes this a totally ineffective exchange site compared to the favorable large ratio of surface area to vessel flow found in capillary beds. It is thus impossible for any sizable exchanges of water or solute to occur between fetus and amniotic fluid by this pathway.

Measurement of water exchange between amniotic fluid and adjacent compartments by isotopic water fluxes.[11, 12] Perhaps the most consistently misunderstood studies of amniotic fluid physiology involve the measurement of isotopic water transfer between mother, fetus, and amniotic fluid and calculation of net water movement in vivo between these spaces from these observations by means of complex compartmental analysis equations. It is quite probable that no accurate estimate of in vivo water movement can be obtained from such studies. Though elegant in design and carefully performed, this human investigation provides an indication of in vivo pathways and relative permeabilities of these exchange sites to isotopic water but does not reliably measure net transfer of bulk water. To clearly understand why this is so, consider the simple example in Fig. 1. If the net movement of water from Side 1 to Side 2 is to be measured from the diffusion of tracer water (k_1), the unidirectional flux of labeled water in both directions [Side 1 → Side 2 (k_1), Side 2 → Side 1 (k_2)] must be measured and the algebraic sum ($k_1 - k_2$) or net flux must be calculated.

The unidirectional flux or diffusion of any compound will be proportional to the total concentration, labeled and unlabeled, of that compound on one side of a permeable membrane. In the case of solvent water, the molar concentration of this molecule is 55.6 moles/L of water. Thus, the transfer of isotopic water by simple diffusion in a direction from Side 1 to Side 2 (k_1) will be proportional to 55.6 moles (the total concentration per liter of labeled and unlabeled water), with the assumption that the labeled water acts exactly like the unlabeled molecule. However, almost all water molecules diffusing from Side 1 → Side 2 will be replaced on a one-for-one basis by an identical molecule diffusing in the opposite direction from Side 2 → Side 1. If a second species of labeled water is introduced on Side 2, the diffusion of this labeled water from Side 2 → Side 1 (k_2) will also be proportional to 55.6 moles/L. If it is assumed that the water volumes and concentrations are equal on the two sides of the permeable membrane, the unidirectional fluxes will be equal and the bidirectional flux will be 0, indicating no net transfer of solvent water. For the purposes of measuring possible in vivo water shifts, consider the creation of a 10 milliosmolar osmotic pressure difference across the membrane (a significant in vivo gradient) by addition of an ideal nonpermeable solute to Side 1. Now the movement of water from Side 1 → Side 2 is no longer proportional to 55.6 moles but very slightly reduced, since the addition of 10 mmoles of solute has reduced the water concentration to approximately 55.59 moles/L of solution. Thus, the unidirectional flux of water Side 1 → Side 2 (k_1) is now reduced by one part in 5,560 below the unidirectional flux in the opposite direction, which is still proportional to the molar concentration of pure solvent water or 55.6 moles. The algebraic sum of these fluxes ($k_1 - k_2$) or the net flux will no longer be zero but will be 1/5,560 of the unidirectional flux and in a direction Side 2 → Side 1, representing the net movement of water in response to an osmotic gradient of 10 mOsm/kg of water. Consider that most body fluids (plasma, interstitial fluid, etc.) have a total solute concentration of approximately 280 mOsm/kg of water. Thus, the water concentration of these fluids is in excess of 55.3 moles/L and the net movement of water or net flux between fluid compartments containing biological fluids in response to a gradient of 10 mOsm/kg of water will represent only approximately 1/5,530 of the unidirectional fluxes.

The very important consequence of this long explanation is that a sizable osmotic transfer of water in response to a very large physiologic gradient (i.e., 10 mOsm/kg of water) would be represented by an extremely small difference in the unidirectional fluxes of isotopic water. This is because of huge steady-state unidirectional fluxes of this molecule since solvent water is the major component of most biological solutions. Therefore, attempts to measure or quantitate in vivo water movement by measuring the transfer of isotopic water between maternal, fetal, and amniotic fluid compartments is very likely impossible. The precision required is clearly far beyond the limits of any current experimental technique. Such studies, therefore, may provide a rough indication of permeability of various tissue layers surrounding the amniotic space to the diffusion of isotopic water in response to a gradient of this labeled molecule but cannot accurately measure net water movement between mother, fetus, and amniotic fluid.

Basic biological mechanisms involved in water transfer

Fundamental to any discussion of fluid dynamics involving the amniotic cavity is a consideration of currently accepted concepts of water transfer in biology. There is essentially no evidence in any part of the body for the active transfer or secretion of water. Water moves across body membranes in response to hydrostatic or osmotic gradients and in the absence of these chemical potential forces no water transfer takes place.

"Bulk flow." In the presence of such gradients water moves across multicellular porous tissue layers such as amnion and chorion by a nondiffusional process known as "bulk flow." Other terms for the same process include laminar or Poiseuille flow and refer to the movement of solvent water in bulk form (similar to flow down a pipe) through water-filled extracellular channels of a porous multicellular membrane in response to a hydrostatic or osmotic gradient. The net movement of water by this process is linear with gradient but exceeds the rate of tracer water diffusion across chorion and amnion by 100-fold to 200-fold.[5] The rate of water transfer is, therefore, accelerated when movement occurs in bulk or solvent form in response to a chemical potential gradient. Such a bulk flow is nondiffusional but does not involve an energy-requiring or active transport mechanism. The passive movement of water molecules becomes augmented when the membrane contains pores or channels wherein the water exists in solvent form. Conversely, the transfer of water across a nonporous membrane (e.g., artificial lipid bilayer or individual cell plasma membrane) in response to an osmotic or hydrostatic gradient occurs at a rate equivalent to simple diffusion.

Table I. Reflection coefficients (σ) measured for partially penetrating solutes across human placental tissue

Solute	*Amnion*	*Chorion*
Urea	0.02	0.01
NaCl	0.04	0.02
Glucose	0.06	0.05
Sucrose	0.07	0.05

The large bulk flows across human placental tissue are determined by measurement of volume changes on the two sides of the membrane in response to an osmotic or hydrostatic gradient. Isotopic water diffusion rates are measured by the net transfer of labeled molecule across this tissue in the absence of changes in solvent volume.

"Leaky or partially semipermeable membrane." Amniotic and chorionic tissue are highly permeable to water. Tracer water diffuses across this tissue approximately 30% as fast as it would diffuse through an equivalent layer of pure solvent water and osmotic or bulk flow transfer of water occurs at 100 to 200 times the tracer water diffusion rate. Since these tissue layers are totally impermeable to many compounds in excess of 1,000 molecular weight, they conform to an ideal semipermeable barrier in regard to these nonpermeating solutes and the osmotic effectiveness of such solutes is described by the van't Hoff equation $\pi = mRT$ (a reasonable approximation of this equation for dilute solutions), where $\pi =$ osmotic pressure (atmospheres), m = molar concentration of solute (moles of solute/kg of water, R = gas constant, and T = absolute temperature (°K).

Many smaller compounds (urea, glucose, NaCl, etc.) diffuse readily across chorion and amnion but still exert some small osmotic force.[5] In this case, placental tissue does not discriminate perfectly between solute and solvent but is partially permeable or leaky to smaller solute molecules. These solutes may diffuse across the placenta in large amounts; however, since they do not cross as rapidly as water, they still exert some small osmotic force. The imperfect ability of the placental tissue to discriminate between solute and solvent and thus the partial osmotic effectiveness of such solutes is described by adding a term (σ) known as the Staverman or reflection coefficient to the van't Hoff expression (Table I). Thus, $\pi = \sigma mRT$ indicates the

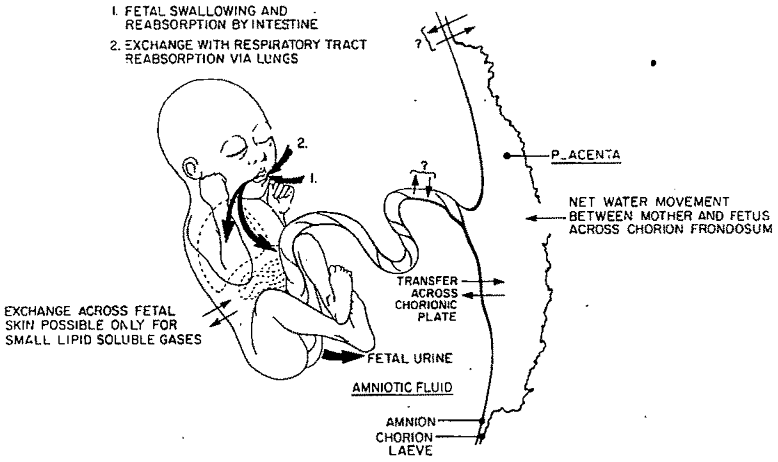
1. FETAL SWALLOWING AND REABSORPTION BY INTESTINE
2. EXCHANGE WITH RESPIRATORY TRACT REABSORPTION VIA LUNGS
?
2.
1.
PLACENTA
?
NET WATER MOVEMENT BETWEEN MOTHER AND FETUS ACROSS CHORION FRONDOSUM
TRANSFER ACROSS CHORIONIC PLATE
EXCHANGE ACROSS FETAL SKIN POSSIBLE ONLY FOR SMALL LIPID SOLUBLE GASES
FETAL URINE
AMNIOTIC FLUID
AMNION
CHORION LAEVE

Fig. 2. Current evidence indicates that significant exchanges of water and solute take place between amniotic fluid and fetus near term by several pathways: (1) fetal swallowing, (2) fetal urination, and (3) within the fetal respiratory tract where the alveolar capillary bed is perfused by amniotic fluid transported in and out by respiratory movements. Under certain conditions transfer of water and solute probably also occurs across the chorionic plate between the fetal circulation and amniotic fluid. There is also evidence to suggest possible transfer of highly lipid-soluble compounds of small molecular weight across fetal skin. It is unlikely that any significant exchange of solute and water occurs across the umbilical cord or across the chorion laeve and amnion away from the discoid placenta. The major pathway for total intrauterine water accumulation in pregnancy is across the chorion frondosum between the maternal and fetal compartments. In the third trimester, intrauterine water increases by approximately 30 to 40 ml per day. Presumably this net water transfer occurs from the mother in response to very small intermittent chemical potential gradients.

osmotic force for a small, partially penetrating molecule where σ indicates the reflection coeficient for that molecule.

The σ for large macromolecules such as albumin, which exert an ideal osmotic force, would be 1.0 where the membrane discriminates perfectly or is totally impermeable to the solute while highly permeable to solvent water. In contrast, urea crosses these membranes rapidly but has a σ of 0.02 (Table I), indicating that this solute exerts 2% of the ideal osmotic effect of a nonpenetrating macromolecule per unit of concentration difference across this tissue.

The partially semipermeable nature of human placental tissue assumes great practical significance when consideration is given to net intrauterine water accumulation in pregnancy. It has been estimated that approximately 4 L of water (2,800 ml in the fetus, 400 ml in the placenta, and 800 ml in amniotic fluid) accumulate within the human uterus during pregnancy, representing an increase of approximately 30 to 40 ml per day near term. This water requirement far exceeds any intrauterine or fetal metabolic source and is most likely supplied by net transfer across the placenta from mother to fetus. A careful search for transplacental chemical potential gradients responsible for such net water transfer has been unsuccessful since maternal and fetal total solute concentration and colloid osmotic and hydrostatic pressure appear to be roughly equivalent across the placenta. In previous reviews[8, 13] data have been collated on these gradients in detail. However, very small chemical potential gradients present for only a short time across the large placental surface area could furnish these water requirements, and one possible explanation for such undetected gradients derives from consideration of the maternal and fetal concentrations of small partially permeable solutes. It has been shown that water transfer may occur across a semipermeable membrane in a direction opposite to that predicted by gradients in total solute concentration if differing solute species with different reflection coefficients are contained on the two sides of the barrier.[14] Thus, a 10 milliosmolar glucose solution will pull water by osmosis across amnion or chorion laeve from a more concentrated 20 milliosmolar urea solution because glucose crosses this tissue more slowly than urea and thus exerts three times the osmotic force of urea per unit of concentration (as indicated by the larger σ in Table I). Thus, the osmotic effectiveness of biological fluids bathing the two sides of the human placenta is not a colligative property of solutions estimated by total solute concentration but must be calculated from the concentrations and reflection coefficients of individual solutes.

Current experimental techniques are probably inadequate to measure the small or intermittent variations in specific solutes with differing amounts of osmotic effectiveness or, in total solute concentration, colloid osmotic pressure or perhaps even hydrostatic pressure that may be responsible for the normal intrauterine water accumulation in pregnancy. The gradients necessary for the small daily net water transfer involved could easily be too small or intermittent for detection.

Exchange of amniotic fluid solute and water with adjacent compartments

There is strong evidence for significant exchanges of water and solute between amniotic fluid and fetal compartments throughout pregnancy, although the largest amount of data has been obtained in the third trimester or near term. Between 12 and 22 weeks' gestational age, Lind and co-workers[15] showed amniotic fluid sodium and urea concentrations very slightly below fetal plasma levels, although closer to fetal than maternal values. These data are compatible with the overall concept that amniotic fluid very early in pregnancy is roughly equivalent to isotonic extracellular fluid derived from maternal and/or fetal compartments by dialysis across tissue layers largely impermeable to protein. These investigators also obtained hypotonic urine from the fetal bladder as early as 12 weeks, suggesting a contribution to amniotic fluid formation earlier than previously suspected.

Amniotic fluid becomes moderately hypotonic (mean total solute concentration 255 to 260 mOsm/kg of water) near term and is thought to be the product of numerous exchanges with the fetus (Fig. 2). The human fetus is estimated to produce 600 to 800 ml per day of very hypotonic (80 to 140 mOsm/kg of water) urine at term, based on fetal urine production rates (0.14 ml/kg/min) measured in chronic pregnant sheep preparations[16, 18] and also from estimates of human fetal urine production by sonographic techniques.[17] Furthermore, late in pregnancy, fetal urine contains large quantities of creatinine, urea, and conjugated estriol, corresponding to increases in amniotic fluid levels of these solutes.[18, 19] In an investigation of the role of possible excess fetal urine production and hydramnios, Van Otterlo and co-workers[17] found no increase in urine volume accompanying hydramnios in the diabetic pregnancy, but Cassady and co-workers[20] reported definite evidence of increased amniotic fluid glucose and total solute concentrations, presumably of renal origin, in diabetic pregnancy. The fetus swallows between 200 and 450 ml of amniotic fluid per day, based on experiments with radioactive chromium-labeled red blood cells[21] and more recently nonradioactive colloidal gold[22] to estimate fetal fluid deglutition (mean swallowing rate ~ 200 ml/day by the colloidal gold method). Thus, fetal swallowing will remove only about half of the daily urine production and the fluid swallowed will have the normal amniotic total solute concentration of 250 to 260 mOsm/kg of water. Therefore, there is a net daily addition of a sizable quantity of very hypotonic urine which must be reabsorbed via other pathways since the amniotic cavity gains a fluid volume of no more than 10 ml/day in the third trimester and the total solute concentration is always in the previously mentioned normal range.

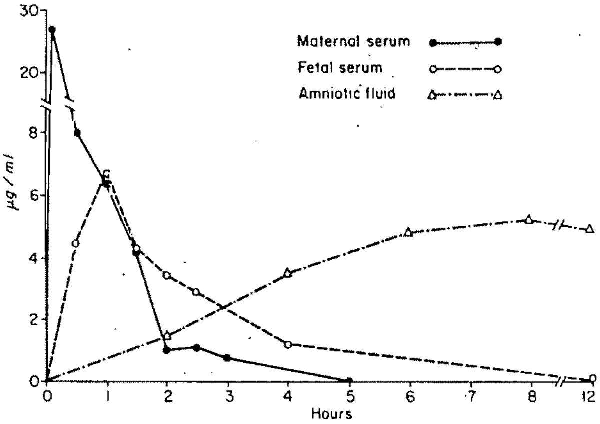

Fig. 3. Comparison of ampicillin concentrations in serum of mother and fetus and in amniotic fluid after maternal intravenous infusion of ampicillin. (From Bray, R. E., Boe, R. W., and Johnson, W. L.: Transfer of ampicillin into fetus and amniotic fluid from maternal plasma in late pregnancy, AM. J. OBSTET. GYNECOL. 96:938, 1966.)

Since fetal plasma is significantly hypertonic to amniotic fluid, a sizable net transfer of fluid from the amniotic to the fetal compartment would result wherever a fetal capillary bed is in proximity to this fluid.

Recent studies by Duenhoelter and Pritchard[23] have indicated a sizable steady rate in and out, or tidal flow (≅600 to 800 ml/day), of amniotic fluid through the fetal lungs in response to active respiratory movements throughout the third trimester. This process would presumably introduce hypotonic amniotic fluid to a large alveolar capillary bed surface area and a daily net reabsorption of fluid could be expected to take place. Furthermore, the association between congenital anomalies or tumors (e.g., tracheal aplasia, nasopharyngeal teratoma, pulmonary hamartoma, diaphrag-

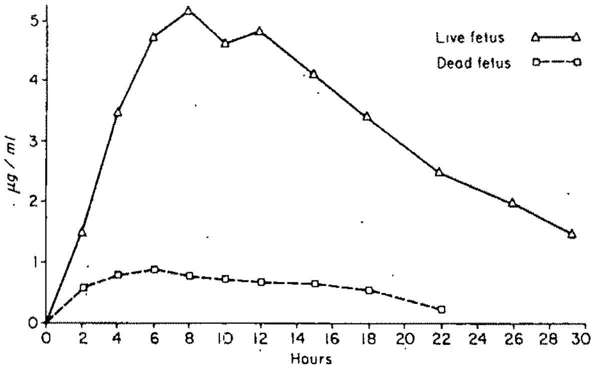

Fig. 4. Comparison of ampicillin concentrations in amniotic fluid of pregnancies with a living fetus and following intrauterine fetal death. (From Bray, R. E., Boe, R. W., and Johnson, W. L.: Transfer of ampicillin into fetus and amniotic fluid from maternal plasma in late pregnancy, Am. J. Obstet. Gynecol. 96:938, 1966.)

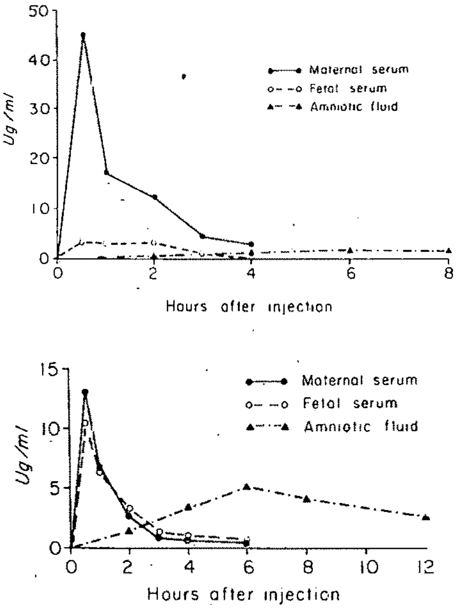

Fig. 5. Concentration of dicloxacillin *(top)* and methicillin *(bottom)* in maternal and fetal serum and amniotic fluid following intravenous infusion in mother. Each patient received 500 mg of the respective antibiotic over a 10- to 15-minute period. Conclusion of infusion is designated as zero time. Methicillin has a molecular weight of 402 and is 40% protein bound. Dicloxicillin has a molecular weight of 510 and is 96% protein bound. (From Depp, R., Kind, A. C., Kirby, W. M. M., and Johnson, W. L.: Transplacental passage of methicillin and dicloxacillin into the fetus and amniotic fluid, Am. J. Obstet. Gynecol. 107:1054, 1970.)

matic hernia) which interfere with fetal breathing movements and hydramnios is recognized.[24–28] Therefore, although previous discussions of amniotic fluid physiology often considered the fetal respiratory tract as a possible site of fluid production, it would appear from the above-cited evidence that this organ system plays a major role in the net reabsorption of water from this cavity.

The large fetal skin capillary bed may serve as a site for amniotic water and solute exchange in early pregnancy but after keratinization of this layer at about 24 to 26 weeks' gestational age, this tissue becomes very impermeable to isotopic water and most solutes.[29] However, even keratinized skin may remain partially permeable to small, highly lipid-soluble compounds such as carbon dioxide and oxygen. Adult skin has been shown to be significantly permeable to oxygen.[30] Mean amniotic fluid Po_2 (11 to 12 mm Hg) and Pco_2 (≅ 50 mm Hg) values late in pregnancy are respectively slightly lower and slightly higher than fetal umbilical artery values, indicating equilibration of these gases with the fetal compartment.[31] Changes in amniotic fluid Pco_2 closely paralleled changes in fetal Pco_2 in the rhesus monkey.[32] Therefore, amniotic fluid levels of those small, highly lipid-soluble compounds probably reflect rapid equilibration of these gases between the fetus and amniotic fluid compared to other solutes, perhaps across exchange surfaces such as fetal skin.

An additional site for possible exchange of water and solute between amniotic fluid and fetus would be across the fetal surface of the discoid placenta, that is, across the amnion and chorionic plate from the placental capillary bed. Abramovich and Page[10] have shown that water, sodium, chloride, urea, and creatinine readily cross this exchange surface. The association in late pregnancy between hydrops fetalis (immunologic or nonimmunologic),[33, 34] a large edematous placenta, and hydramnios implies that a fetal pathologic condition involving an increased central venous pressure and thus extravasation of excess fluid into fetal and placental interstitial spaces eventually results in deposits of excess fluid in the amniotic space. Since the transfer of a significant amount of water across keratinized skin remains unlikely, a pathway from the placental capillary bed across the fetal surface of the placenta is strongly suggested to explain these related findings. The only possible explanation would be an increased fetal urinary output and since many of these hydropic babies also have some degree of cardiac failure, this seems unlikely as a consistent mechanism.

Prolactin has been reported[35] in preliminary studies to reduce permeability of human amnion in vitro to water transfer from the fetal to the maternal side. These findings together with a significant reduction in normally very high amniotic fluid prolactin values associated with hydramnios have resulted in speculation that prolactin may be active in the regulation of human amniotic fluid volume and composition. This hypothesis awaits further confirmation, however, since a significant exchange of amniotic fluid water and solute with the fetal and/or the maternal compartment across the chorion laeve and amnion away from the discoid placenta appears highly improbable in view of the sparse vascularity in this region.

Maternal drug administration

From the preceding discussion, it is clear that particularly in the latter part of pregnancy, amniotic fluid is almost completely the product of sizable, rapid, and consistent steady-state exchanges with the fetus by numerous pathways. For the most part, maternal–amniotic fluid exchange in late pregnancy occurs only indirectly through the fetus. Confirmation of this concept comes from a review of the distribution of various drugs given to the mother in pregnancy. For example, ampicillin (molecular weight 349), a commonly used antibiotic, when given in a bolus intravenous dose to the mother, appears in peak concentrations within the fetal compartment in 30 to 60 minutes (Fig. 3) and in significant quantities within the amniotic fluid in 2 hours, to reach peak levels at 6 to 12 hours.[36] However, only a very small amount of this drug reached the amniotic fluid following an equivalent maternal dosage when the fetus was dead (Fig. 4), thus reinforcing the concept that most compounds must be transferred from mother to fetus in order to gain entry into the amniotic space. Dicloxacillin, another similar-sized (molecular weight 510), water-soluble antibiotic, diffuses slowly (because of 96% binding to plasma proteins) and in small amounts to the fetus (Fig. 5) and, therefore, only very small amounts of the drug appear in amniotic fluid.[37]

From the preceding discussion of pathways for fetal–amniotic fluid exchange, one can postulate several possible mechanisms for drug distribution from maternal to intrauterine compartments. Highly lipid-soluble compounds of small molecular weight such as oxygen, carbon dioxide, and nitrous oxide cross quickly (peak fetal concentrations within 2 to 3 minutes) to the fetus and probably also equilibrate in the amniotic cavity within a very few minutes by diffusion across the fetal skin. Evidence for this includes studies in primates where increases in fetal P_{CO_2} were accompanied by parallel increases in amniotic fluid P_{CO_2} within 2 to 3 minutes.[32] Rapid increases in fetal nitrous oxide concentrations following maternal inhalation were accompanied by similar increases in amniotic and allantoic fluid concentrations of this substance in sheep.[38] The rapid transfer of larger lipid-soluble compounds such as phenobarbital in dogs[39] and meperidine in humans[40] from mother to fetus and amniotic fluid suggests that these substances may also diffuse across fetal skin or the fetal side of the placenta from fetal capillary beds. Small but significant amniotic fluid concentrations of phenobarbital and meperidine were detected within 10 to 30 minutes of maternal intravenous infusion. However, within 2 to 3 hours, amniotic fluid meperidine concentrations significantly exceeded maternal and fetal levels (Fig. 6), indicating that sizable amounts of this compound were excreted in fetal urine and were unable to back-diffuse to equilibrate with fetal levels. Therefore, diffusion across skin or other exchange surfaces may play a minor role in entry of this substance into the amniotic fluid.

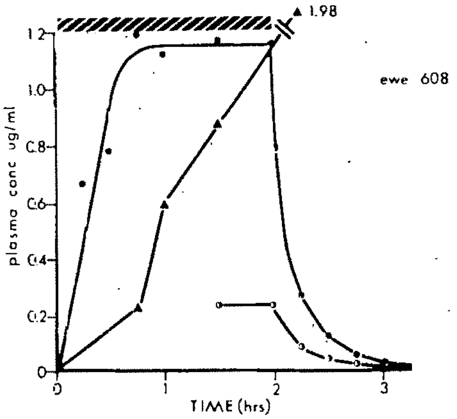

Fig. 6. Concentration of meperidine in maternal sheep plasma (●), fetal plasma (◑), and amniotic fluid (▲) during and after a continuous infusion of meperidine to the mother (0.06 mg/kg for 2 hours). (From Szeto, H. H., Mann, L. I., Bhakthavathsalan, A., Liu, M., and Inturrisi, C. E.: Meperidine pharmacokinetics in the maternal-fetal unit, J. Pharmacol. Exp. Ther. 206:448, 1978.)

Thus, several general mechanisms for maternal–fetal–amniotic fluid distribution of drugs can be postulated.

1. Small, highly lipid-soluble compounds diffuse rapidly to the fetus, reaching peak concentrations within 2 to 3 minutes, but also appear quickly in amniotic fluid probably by diffusion across fetal skin or the fetal surface of the placenta (Fig. 7). However, since these compounds appear to readily diffuse between the fetus and amniotic fluid, their concentration in amniotic fluid should parallel and not exceed fetal levels.

2. Larger-sized, water-soluble, lipid-insoluble compounds (e.g., antibiotics, mannitol) cross more slowly to the fetus but reach significant peak levels 30 to 60 minutes following maternal infusion (Fig. 3). These compounds can also be detected in amniotic fluid within 20 minutes, most likely secondary to fetal urine production (Fig. 8), but reach peak levels much higher

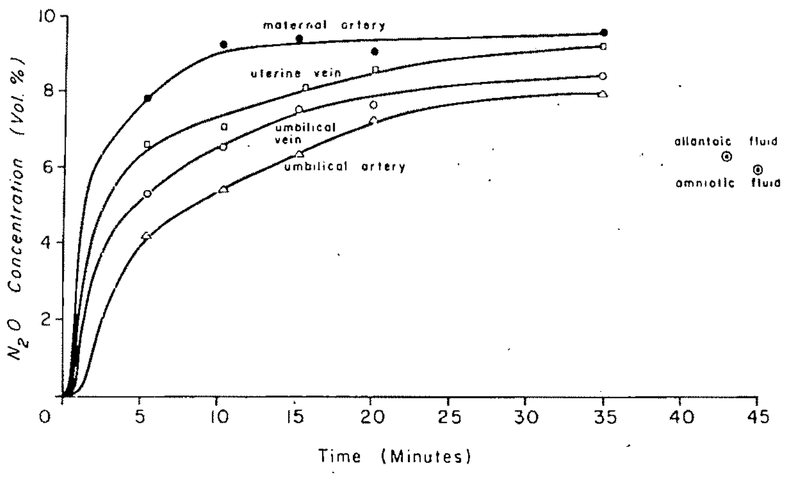

Fig. 7. Maternal and fetal nitrous oxide concentrations during maternal N_2O inhalation in sheep pregnancy at 123 days. N_2O concentration in allantoic and amniotic fluids also is shown. (From Blechner, J. N., Makowski, E. L., Cotter, J. R., Meschia, G., and Barron, D. H.: Nitrous oxide transfer from mother to fetus in sheep and goats, AM. J. OBSTET. GYNECOL. 105:368, 1969.)

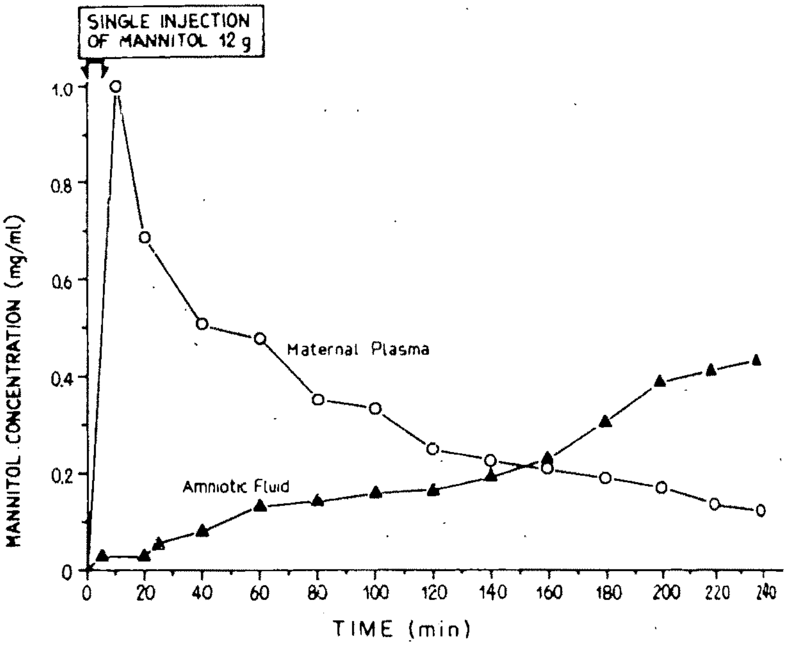

Fig. 8. Concentrations of mannitol in amniotic fluid and maternal plasma after a single intravenous injection of this substance to a pregnant woman at term with a live fetus. The concentration of mannitol in amniotic fluid increased with time, reaching values higher than those found in maternal plasma from 160 to 240 minutes. (From Basso, A., Fernandez, A., Althabe, O., Sabini, G., Piriz, H., and Belitzky, R.: Passage of mannitol from mother to amniotic fluid and fetus, Obstet. Gynecol. 49:628, 1977.)

than fetal or maternal plasma levels at 4 to 24 hours following maternal infusion (Figs. 3 and 5). The high amniotic fluid levels of these water-soluble compounds correspond to high fetal urine levels and a trapping effect which occurs after the introduction of these compounds into amniotic fluid. Since fetal skin in late pregnancy is quite impermeable to these substances, back-diffusion or equilibration will be very slow after amniotic cavity entry concentrated in fetal urine, thus, amniotic fluid values of these solutes significantly above maternal and fetal levels will occur. Numerous examples can be cited for water-soluble, lipid-insoluble com-

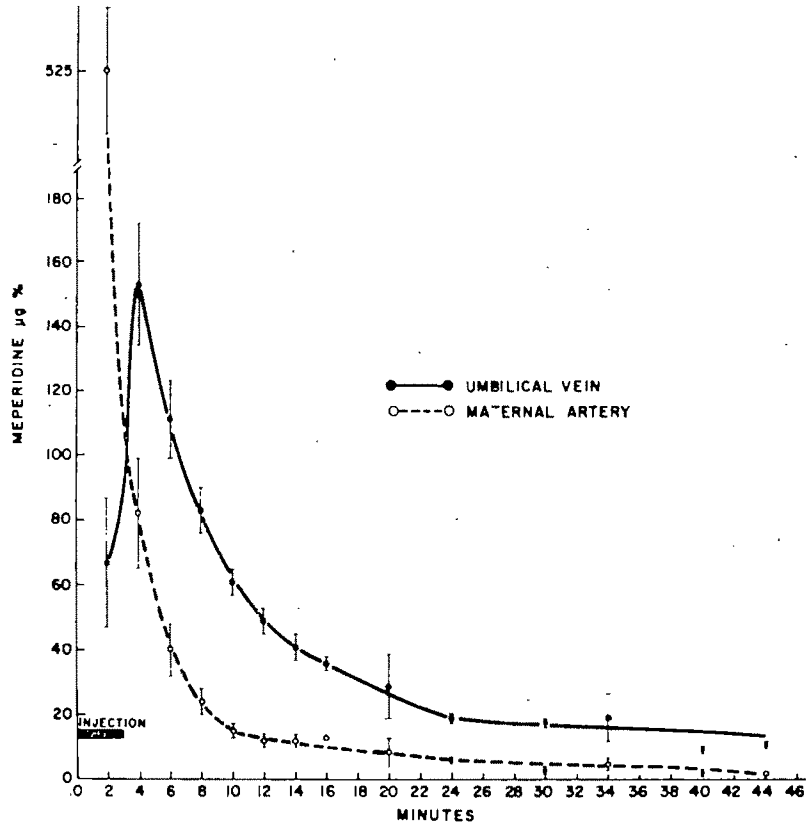

Fig. 9. Mean + 1 SE of maternal sheep arterial and fetal umbilical vein meperidine HCl levels following maternal intravenous infusion. (From Shier, R. W., Sprague, A. D., and Dilts, P. V., Jr.: Placental transfer of meperidine HCl. Part II, AM. J. OBSTET. GYNECOL. 115:556, 1973.)

pounds of intermediate molecular size (molecular weight 200 to 600) that cross rather slowly but in significant quantities to the fetus and eventually achieve sizable amniotic fluid levels by entry in fetal urine. Evidence exists for similar fetal and amniotic fluid distribution of methicillin (Fig. 5), penicillin,[41] sulfonamides,[42] some cephalosporins,[43] tetracycline,[44] kanamycin,[45] and gentamicin,[46] in addition to ampicillin.

3. Some compounds such as the narcotics and analgesics are highly lipid soluble but have a larger molecular size (molecular weight 150 to 300) than the respiratory or inert gases. These solutes may gain access to amniotic fluid by a combination of events, but, in general, rapid transfer to the fetus followed by slower entry into the amniotic cavity and levels significantly above fetal and maternal values indicate addition of this solute in fetal urine and inability to back-diffuse to the fetal compartment. Evidence in the chronic sheep model of high meperidine concentrations in amniotic fluid illustrate this concept[40] (Fig. 6). Thus, an intermediate rate of appearance in amniotic fluid for these compounds could be postulated. Such highly lipid-soluble agents would diffuse rapidly from mother to fetus to achieve peak concentrations within 2 to 3 minutes (Fig. 9) but would probably cross in significant quantities to amniotic fluid in fetal urine more slowly than the smaller lipid-soluble gas molecules, which may diffuse directly across fetal skin.

4. Finally, a fourth general category of fetal–amniotic fluid drug distribution could be described for compounds that cross in significant quantities to the fetus but are largely metabolized in the fetal liver and thus appear in fetal urine and amniotic fluid in only small amounts. Placental Dilantin transfer in the goat (Fig. 10) illustrates this concept. This intermediate-sized, lipid-insoluble compound diffuses readily, although not rapidly, to the fetus but cannot diffuse across fetal skin and is largely metabolized in the fetal liver,[47] so that only small quantities appear in fetal urine and amniotic fluid.

Thus, most available evidence indicates that there is little direct maternal exchange of water and solutes with amniotic fluid at term in the human and that if a compound does not cross to the fetus it will not appear in amniotic fluid. Data collected from placental an-

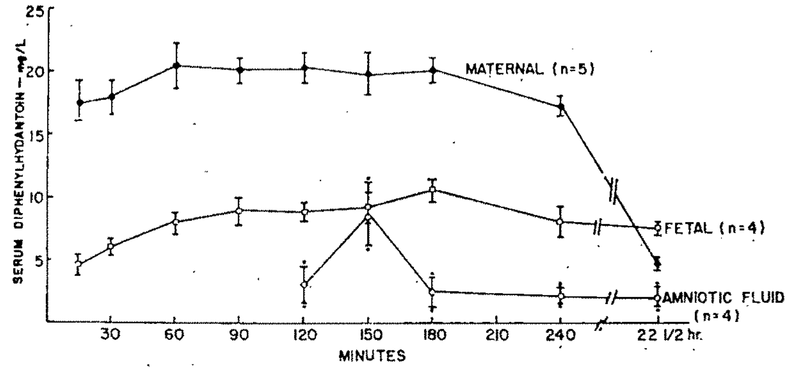

Fig. 10. Time course of maternal and fetal serum and amniotic fluid diphenylhydantoin levels during constant infusion of diphenylhydantoin (2.92 mg/kg/hr) following a loading dose of 7.3 mg/kg. The infusion was discontinued at 120 minutes. (From Shoeman, D. W., Kauffman, R. E., Azarnoff, D. L., and Boulos, B. M.: Placental transfer of diphenylhydantoin in the goat, Biochem. Pharmacol. 21:1237, 1972.)

tibiotic transfer in humans[36] and nitrous oxide transfer in sheep[38] strongly support this concept which, of course, coincides well with the view that amniotic fluid near term is principally the product of exchanges with the fetal compartment.

Amniotic fluid drug infusion

Up until this point, we have reviewed maternal–fetal–amniotic fluid drug distribution in terms of maternal administration. However, the intriguing possibility that amniotic fluid infusion of pharmacologic agents could provide a mechanism for fetal medication in utero has led to several promising but very preliminary studies. Seeds and co-workers[48] demonstrated a significant elevation in fetal pH and bicarbonate concentration within a few minutes following intra-amniotic infusion of sodium bicarbonate in primates. A somewhat inconsistent response in these experiments suggested that perhaps fetal swallowing played a major role in the transfer of this solute from amniotic fluid to fetus. Szeto and co-workers[40] measured the rapid transfer of meperidine to both fetal and maternal compartments following intra-amniotic infusion in the chronic sheep model. Peak drug levels in the fetus were obtained within 45 minutes. Sack and co-workers[49] have also demonstrated significant transfer in sheep of radiolabeled thyroxine and triiodothyronine to the fetus within 24 hours of amniotic fluid infusion. Such findings, if confirmed in humans, would have significant relevance since thyroxine crosses the human placenta very slowly and attempts to provide additional fetal thyroxine by means of maternal administration (to protect the fetal thyroid gland from goiter formation during maternal antithyroid drug administration) have been unsuccessful. Additional studies in sheep[50] have provided evidence for significant fetal transfer of the highly lipid-soluble beta-blocking drug propanolol within 15 minutes of amniotic fluid infusion while lipid-insoluble sotalol crossed slowly and in smaller amounts.

Such data suggest that water-soluble, lipid-insoluble compounds infused into the amniotic sac may gain entry into the fetal compartment in a delayed, somewhat unpredictable fashion by fetal swallowing, whereas lipid-soluble compounds introduced into this space cross to the fetus more rapidly and in larger amounts. This facilitated transfer of the lipid-soluble solutes occurs via unknown pathways, but one can speculate diffusion across the fetal surface of the placenta or fetal skin may be involved.

Future diagnostic uses of amniotic fluid

Additional diagnostic use of amniotic fluid may result from a better understanding of the physiology of this compartment. Fetal scalp blood pH has been successfully used as an indicator of fetal status. Studies in primates indicated that elevated Pco_2 and decreased pH during fetal distress were rapidly accompanied by parallel changes in amniotic fluid values.[32]

Physically dissolved carbon dioxide appears to equilibrate quickly between mother, fetus, and amniotic fluid while the bicarbonate anion crosses very slowly between the three compartments. Therefore, changes in fetal pH secondary to Pco_2 fluctuations would be rapidly transmitted to the amniotic fluid in most circumstances, while fetal pH alterations accompanying changes in bicarbonate concentration, would be poorly reflected in this space. To date, however, correlations between amniotic fluid pH and fetal status at birth in the human have been inconsistent.[31]

Better evidence exists for a correlation between increased amniotic fluid total solute concentration and fetal distress in the human. Increases in amniotic fluid total solute have been measured in cases of fetal death in utero or chronic fetal distress[51] and conform to evidence in the chronic sheep preparation of a consistent increase in urinary total solute and a decrease in volume during episodes of fetal hypoxia and acidosis.[52]

Increases in amniotic fluid glucose[20] paralleled the fetal accumulation of glucose in the diabetic pregnancy and recent evidence suggests that amniotic fluid concentrations of insulin[53] and β-hydroxybutyrate[54] may also correlate with fetal values, reflecting fetal status in this important high-risk pregnancy complication.

Such preliminary data suggest the possibility of many additional uses of amniotic fluid in the future to assess acute and chronic fetal status.

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CORRESPONDENCE

Outcome of pregnancy subsequent to induced abortion

To the Editors:

In their recent article, Dr. Stephen C. Schoenbaum and associates (AM. J. OBSTET. GYNECOL. 136:19, 1980) summarized the records of 5,003 deliveries and concluded that "women with a single prior induced abortion have no increased risk of poor outcome of the next pregnancy after 27 weeks' gestation." In Table III of their analysis they actually demonstrate a 23% increase in the rate of premature labor among those primiparous women who had a previous induced abortion as compared to those who did not (3.7% versus 3.0%) but dismiss the difference as statistically insignificant. In the same spirit they reassure us that the conclusions of Moriyama and Hirokawa[1] were erroneous by stating: "Among women who delivered prematurely and who had had no prior live birth, 22 of 76 (28.9%) had had a preceding induced abortion; whereas, among women who delivered at term who had had no prior live birth, 817 of 3,962 (20.6%) had had a preceding induced abortion . . . None of these differences is statistically significant."

In the course of a recent review,[2] I had the opportunity of analyzing dozens of articles from overseas; most of which provided strong support on behalf of the existence of a cause-effect relation between induced first-trimester abortion, on the one hand, and premature birth in subsequent gestations, on the other. Interruption of the first pregnancy appeared particularly conducive to unfavorable future reproductive performance according to the testimony of these studies. In the light of this information I respectfully submit that what Schoenbaum and associates and Moriyama and Hirokawa have demonstrated was an increased incidence of premature labor following artificial interruption of a woman's first gestation, suggesting a "possible" or "probable" cause-effect relation in the absence of a statistically significant difference and not the absence of an increased risk, as the authors suggested.

Since the medical profession has accepted therapeutic abortion as a legitimate method of birth control, numerous articles have been published in the American literature indicating that no "statistically significant" increase of subsequent unfavorable reproductive outcome could be documented on the ground of the almost invariably limited data that the authors had reviewed. These results have usually been presented as evidence indicating the nonexistence of such effect. Well in keeping with this fast-developing "tradition," Schoenbaum and associates chose to interpret the absence of significance at the 95% confidence limits as evidence of lack of correlation between induced abortion and prematurity rather than as an indication of the fact that their data base was too small for a definite conclusion.

It appears to this writer that physicians, before reassuring their patients about the innocent nature of abortion, should be able to refer to statistical data which prove "within 95% confidence limits" that abortion *cannot* have an unfavorable impact upon their future reproductive life. The assumption that the risks of abortion must be proved with conclusive statistical data represents a preconceived prejudice. What requires unequivocal proof is that it does not.

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Reply to Dr. Iffy

To the Editors:

Let us first get the data straight. Table III in our paper shows that 6.7% of the offspring of primigravid women were born at less than 37 weeks' gestation while only 4.8% of the offspring of secundigravid women whose first pregnancy terminated with an induced abortion were born prematurely. The corresponding percentages of birth weights less than 2,500 gm were 8.2 versus 6.8. These figures simply are not consistent with the notion that there was excessive prematurity for secundigravid women with a prior induced abortion.

Dr. Iffy has chosen to focus on other data in Table III, the percentages of women who had an admission for premature labor (3.0 for primigravid women versus 3.7 for secundigravid women with a prior induced abortion). These were prepartum admissions in which the discharge diagnosis was premature labor, not admissions for premature delivery. Dr. Iffy has ignored the data in the same table indicating that an even higher percentage of secundigravid women whose first pregnancy ended in a live birth also had an admission for premature labor (3.9%). This group by no other criterion demonstrated excessive prematurity.

Though Dr. Iffy quoted some of our comments about the study by Moriyama and Hirokawa, he selectively omitted the fact that in the same study 22% of women with two living children who were delivered prematurely versus 41% of women with two living children who were delivered at term had had an immediately preceding induced abortion. We doubt that Dr. Iffy or anyone else would conclude from this information that women with two living children should then have an induced abortion to decrease their chance of prematurity in the next pregnancy. The study by Moriyama and Hirokawa is simply too small to state conclusively anything about the relationship between prior induced abortion and subsequent prematurity.

Dr. Iffy does make a very important point with which we totally agree. Stated in different terms from those in his letter, it is crucial to consider the power of a study to demonstrate an association. The careful reader of our paper will note that we have raised this issue. The confidence interval around an estimate of risk is an expression of the power of the study to demonstrate a difference of a certain magnitude. In our study the relative risk of having a baby before 37 weeks was 0.7 for women whose first pregnancy led to an induced abortion, and the 95% confidence limits were 0.4 to 1.4. Thus, the data in our study suggest that there is only a 2.5% chance that the true risk exceeds 1.4. To obtain a narrower confidence limit would require an even larger study.

We are currently collecting a substantially larger data set and will be pleased to report the new estimate of relative risk and its confidence interval when the study is completed and analyzed. In the meantime, women contemplating having their first abortion have available to them two recent studies from the United States, neither of which shows an increased risk of prematurity in the next pregnancy.

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Treatment of pelvic inflammatory disease

To the Editors:

In a recent issue of *The Journal of the American Medical Association* (243:414, 1980), Dr. Franz Joseph Ingelfinger, noted gastroenterologist and editor emeritus of *The New England Journal of Medicine,* was quoted as saying that he finds offensive "the increased number of authors per paper" and that "some of the better giveaways, not throwaways, are more informative, more professional, more lively than the poorer standard journals." An example of what Dr. Ingelfinger was alluding to has recently been published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY (136:179, 1980), as evidenced by the article, entitled "The microbiology and therapy of acute pelvic inflammatory disease in hospitalized patients," authored by Thompson, Hager, Wong, Lopez, Ramsey, Allen, Stargel, Thornsberry, Benigno, Thompson, and Shulman. Although there is definite need for investigation into the pathogenesis and treatment of pelvic inflammatory disease, this work adds little to that knowledge.

The information obtained on the organisms isolated from various sites and the determination of the minimal inhibitory concentrations are to be commended, but I do not believe the authors' conclusions can be justified with the information as presented. They stated that there was no difference in disease outcome with either parenteral penicillin and gentamicin or oral amoxicillin and that the initial detection of an abscess does not contraindicate a trial of medical therapy, since most will resolve on antibiotics alone.

In the text, the authors reported that the patients on parenteral penicillin and gentamicin were switched "as soon as possible to ampicillin 2 gm by mouth" while the other group continued on amoxicillin but in a reduced dose. It is difficult for the reader to determine the length of therapy with penicillin and gentamicin for each patient, as this is not clearly stated. It is possible that the actual comparison in this study may be ampicillin to amoxicillin, with the expectation that both drugs would result in similar patient responses.

I question the authors as to how they determined that the patients had a pelvic abscess as they made the statement that "most will resolve on antibiotics alone." In 25 of the 30 patients studied, the pelvis was not directly visualized. I am amazed at the ability of the gynecologic resident to determine accurately which patient had a pelvic abscess in the face of acute pelvic inflammatory disease with abdominal tenderness, guarding, and adnexal tenderness.

The authors seem to disregard the fact that there is much more to the treatment of pelvic inflammatory disease than following the response of temperature, white blood cell count, and a left shift. If one wishes to preserve a patient's fertility, it may be that aggressive parenteral antibiotic therapy will be required to prevent the long-term sequelae of pelvic inflammatory disease.

It is to be noted that the legends for Figs. 1 and 2 have been inadvertently reversed.

Although the authors have attempted to give the practitioner a clearer view of the treatment of pelvic inflammatory disease, they have only further muddied the waters surrounding this clinical entity. It is hoped that the reader will not jump on the bandwagon of this oral therapy for pelvic inflammatory disease but will continue to use sound medical judgment in treatment of this serious infection.

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Reply to Dr. Weinstein

To the Editors:

All those who contributed substantially to the study authored the paper.

We recognize that to accurately diagnose a pelvic abscess by physical examination during the early stages of acute pelvic inflammatory disease (PID) is difficult because of the abdominal tenderness and guarding. However, every day one of the study team physicians (as stated in the text), all of whom were experienced clinicians, performed pelvic examinations on the patients.

We did not state categorically that either of our regimens is a cure-all for patients with suspected pelvic abscess. We merely noted that some women who probably had an abscess were clinically cured by the time of follow-up examination; the text indicates that all were not cured, but with a substantial number of cures a trial of medical therapy is initially acceptable.

We agree with Dr. Weinstein that there is much more to the treatment of PID than observing the temperature, the white blood cell count, and the differential cell count. We used those as ancillary objective measurements to help demonstrate the similarity between the penicillin/gentamicin and amoxicillin treatment groups. Clearly the major indicator of improvement and the ultimate assessment of cure was clinical response. We attempted to organize this complex response into an objective severity score to allow us to be able to compare patients seen by different physicians and to act as a rough guide of day-to-day improvement in the patient for the individual physician. We do not think the principles of treating infectious diseases change merely because the patient is a woman and has PID; the white blood cell count and differential cell count are still useful ways to evaluate infection.

It is certainly true that effective therapy may be required to prevent infertility but whether this is the same as Dr. Weinstein's "aggressive" therapy is not clear. Merely giving antibiotics for a long time by vein—aggressive therapy—is not always the answer. We have merely stated in our conclusion that the two regimens were equally effective in this study. We make no claims about oral antibiotics as drugs of choice.

The paper speaks for itself about the seriousness of the disease when treatment failures are discussed.

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Major vessel injury during laparoscopy

To the Editors:

Major vessel injury during laparoscopy, as reported by Katz and associates,[1] fortunately is a rare occurrence. It is my opinion that accidents of this sort are almost invariably due to operator error. In all but the most obese patient, it is possible to palpate the aorta up to its point of bifurcation. It is, therefore, a simple matter to insert the needle below this point. The position of the umbilicus relative to the termination of the aorta is an unreliable topographic guide. A technique which has proved helpful to ascertain proper placement is that of using a saline-filled 10 ml syringe barrel attached to the needle with the valve open.[2] Under these circumstances, the room air pressure coupled with the negative pressure within the abdominal cavity during respiration will produce a rapid flow of the saline by gravity alone, giving a good visual end point. The plunger is then inserted into the barrel and aspiration is performed to make sure there is no return of saline, blood, urine, or fecal material.

A second point is that injury to the iliac vessel must necessarily occur if the needle or trocar is placed off the midline. This is contrary to good technique except in those cases where dense midline scar tissue presents a problem. If one inserts the needle well below the aorta, the only fixed vessel that is vulnerable is the middle sacral artery. Finally, the suggestion of the authors that raising the abdominal wall with towel clips will overcome problems is one which produces an illusion of safety. Elevation of the skin does nothing to the peritoneum and only increases the distance over which the needle must travel. It is occasionally possible to elevate manually the entire abdominal wall; this is true only in thin patients who also have lax musculature. Overzealous use of towel clips to elevate the entire abdominal wall may result in pinning the bowel between the points of the instrument. Therefore, the best technique remains one of entry into the abdomen below the bifurcation of the great vessels and aiming toward the sacral concavity.

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2. Corson, S. L.: Use of the laparoscope in the infertile patient, Fertil. Steril. **32**:359, 1979.

To the Editors:

It was a pleasure to see case reports of this major concern of laparoscopists appear from American authors in an American journal. The authors are to be congratulated for overcoming the legal inhibitions of our times and sharing with us their experiences and insights. As Chairman of the Complications Committee of the American Association of Gynecologic Laparoscopists, I have heard of over 100 of such major vessel injuries in the United States and have been dismayed that the physicians concerned were fearful of publishing these occurrences. The article by Katz and associates, is to my knowledge the first such report in the American literature.

In analyzing the probable cause of arterial lacerations, the authors correctly identify the need to elevate the abdominal wall before blind insertion of instruments. This is particularly true in small, nulliparous women, with strong abdominal walls, where the aorta may lie less than an inch below the umbilicus. The technique the authors recommend (raising the abdominal wall with towel clips) is satisfactory, but other techniques are equally useful. Many laparoscopists grasp the lower abdomen and elevate it so as to elevate the umbilicus up and away from the posterior pelvic vessels. Peritoneoscopists have traditionally asked the patient to inhale and "bear down," thus pushing the rectus muscles away from the pelvic vessels with the bowel. Hasson[1] recommended suturing the fascia with strong catgut and pulling on the fascia directly, rather than relying on towel clips which occasionally slip in obese patients.

The Z technique of insertion has the risk which the authors describe—lack of control in the thrust of the trocar, with the resultant lateral insertion into a common iliac artery or vein. This problem, incidently, is also a feature of the insertion of the second-puncture trocar off the midline and above the umbilicus. At least one death has occurred in the United States because of a puncture of the common iliac artery by the second-puncture trocar. The physician inserting these trocars may be unaware of the direction in which the trocar is actually being pointed. It is useful to have a colleague stand at the head or foot of the table with a clear view of the lateral direction in which the primary and secondary trocars are being inserted. A few such observations will correct a physician's inadvertent bad habit of directing a trocar more laterally than he intends and will further minimize the risk of major vessel injury.

I would like to thank the authors for providing this opportunity to comment on this major complication and for suggesting additional ways of minimizing its occurrence.

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REFERENCE

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Pregnancy in a patient with three negative pregnancy tests and hyperprolactinemia

To the Editors:

The current topic in vogue in obstetrics and gynecology is hyperprolactinemia and the various tests to adequately determine the etiology and ultimate treatment of the condition. This case report serves to illustrate that we must continue to think of the simpler, more common diagnosis while we are entangled in the myriad of tests involved to evaluate the condition.

H. M., a 27-year-old woman, had a history of normal menses until January 21, 1980. She was seen in the office on March 20, 1980, complaining of amenorrhea. She denied nausea and vomiting, breast tenderness, urinary frequency, and lassitude. She also denied galactorrhea, weight gain, and hirsutism. Two prior pregnancy tests were negative. Physical examination revealed a slightly soft, normal-sized, anteflexed uterus. A repeat pregnancy test was negative and she was given Provera, 10 mg every day for 5 days, to bring on a flow. The patient returned to the office April 7, 1980, stating that she had no withdrawal bleeding. Pelvic examination was unchanged. A serum sample for determination of prolactin was obtained. On April 10, the patient aborted and underwent dilatation and curettage. The pathology report revealed acutely inflamed necrotic decidua and fragments of gestational endometrium. The prolactin level, reported April 11, was 48.5 ng/ml.

Hyperprolactinemia can occur in a variety of conditions.[1] It is believed to interfere with fertility, and treatment regimens have been devised to inhibit the circulating level of prolactin.[2] However, the most common cause of hyperprolactinemia is pregnancy and this should always be ruled out prior to a full investigation. Perhaps a pelvic sonogram should be obtained in all patients with elevated prolactin levels. In this way an extensive workup with its inherent risks of radiation to the fetus could be avoided.

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Amniotic fluid lipids of patients with sickle cell disease

To the Editors:

This letter is in reply to the comments of Dr. Eric J. Sing (AM. J. OBSTET. GYNECOL. 136:1089, 1980) on the publication of S. K. Das and H. W. Foster, "Amniotic fluid lipids in sickle cell disease," in the January 15, 1980, issue of the JOURNAL, on page 211.

We are pleased that Dr. Sing has expressed an interest in our views on the presence of polyunsaturated fatty acids in amniotic fluid lecithin.[1] In our study, we analyzed total lecithin that included not only surfactant but also nonsurfactant lecithin. Lung surfactant lecithin is mostly of the dipalmitoyl type. It is produced by a complex mechanism involving either a deacylation-reacylation or a deacylation-transacylation process to retailor the fatty acyl moieties of phosphatidylcholine (PC) produced de novo by the cytidine diphosphate choline pathway. PC produced de novo contains unsaturated fatty acids including prostaglandin precursors in the 2-position. It is possible that in sickle cell anemia the auxiliary processes function at a reduced capacity and thereby the unsaturation at the 2-position of PC is preserved. Polyunsaturated fatty acids at the 2-position of PC may thus be precursors of prostaglandins in sickle cell anemia and thereby linked in some way to the respiratory distress syndrome. In regard to the high amounts of phosphatidylserine and phosphatidylethanolamine found in the amniotic fluid of sickle cell patients, we suggest that these lipids may be involved in blood clotting in sickle cell patients since these two lipids are known to be responsible for the thromboplastic activity of platelets.[2]

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ITEMS

Recertification by The American Board of Obstetrics and Gynecology, Inc.

The following doctors were recertified by The American Board of Obstetrics and Gynecology, Inc.

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Hayes, James F., Jr.
Healey, Francis J.
Hennisch, Melvin
Henriksen, C. David
Herning, Howard F.
Herrin, Eugene V.
Herzog, Godofredo M.
Hesse, Charles F.
Hirsch, Nathan B.
Hirsch, Robert D.
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Horwitz, David Alexander
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Huse, William J., II
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Jabamoni, Reena
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Jackson, Neil D.
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Jacobs, Michael L.
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Johnson, Carl E.
Johnson, James S.
Johnson, Russell E.
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Jones, D. E. Darnell
Jones, Donald A.
Juan, Paul L.
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Kachinski, Frank E.
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Kahane, Albert J.
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Kang, Chang-Man
Kanter, Earl
Karten, Gerald J.
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Keating, Garrett M.
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Kelly, William Thomas
Kennison, Robert D.
Kent, Howard L.
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Kesler, Kelvin F.
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King, Albert G.
Kirsch, Bernard
Kirschbaum, Thomas H.
Kitchings, John Taylor, Sr.
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Klay, Leonard John
Klinges, Karl G.
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Krafte, Burton O.
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Lamastra, Philip M.
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Lapid, Louis S.
Laubstein, Melvin B.
Leavitt, Thomas, Jr.
Le Vine, Lawrence
Lee, Frederick Y. L.
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Lemmon, Francis M., Jr.
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Leon, Peter
Levin, Norman
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Li, Chiu Fook
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Limia, Anthony G.

Lin, Hong-Chien
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Loewenberg, Leopold S.
Logan, Bernard
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Lord, E. A. R., Jr.
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Lucas, John R.
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Lundy, Laurence
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Masterson, Byron J.
Matala, Joseph S.
Mathis, James E.
Mattox, John H.
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Minei, Lawrence J.
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Moore, Francis E. Moore, Jr.
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Morley, Buel N.
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Mullick, Swadesh
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Sherman, Alfred I.
Siegelbaum, Harold L.
Silva, Pedro A.
Simes, David L.
Simonson, Emmett D.
Sirin, Kerim Fikret
Sivitz, Jay
Skipton, Robert D.
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Strong, James Doyce, Jr.
Sturman, Herman Getzie
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Susong, William A.
Suter, Max
Sylvester, James A.
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Trief, Oscar
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Zarate, Eugenio A.
Zelnick, Saul
Zivkovic, Dusan M.
Zucca, John L.

Fourth Annual Mid-Winter Symposium in Obstetrics and Gynecology

The Fourth Annual Mid-Winter Symposium in Obstetrics and Gynecology will be held February 25-27, 1981, at the Scottsdale Hilton Hotel, Scottsdale, Arizona. It is cosponsored by the Phoenix Obstetrics and Gynecology Society and the Department of Obstetrics and Gynecology, Maricopa County General Hospital.

For information contact: The Secretary, Phoenix OB/GYN Society, 333 E. Virginia, Suite 222, Phoenix, Arizona 85004.

Twelfth International Congress of Chemotherapy

The Twelfth International Congress of Chemotherapy will be held July 19-24, 1981, in Florence, Italy. Deadline for abstracts: February 15, 1981.

For information write: Secretariat, 12th International Congress of Chemotherapy, Via della Scala, 10, 50123-Florence, Italy.

Clinical Focus: Obstetrics and Gynecology

A program, entitled "Clinical Focus: Obstetrics and Gynecology," sposored by SUNY-Upstate Medical Center, will be held February 15-21, 1981, at the Hilton Inn, Lake Placid, New York.

For more information contact: Program Coordinator, Office of Continuing Education, Upstate Medical Center, State University of New York, 750 East Adams St., Syracuse, New York 13210.

Oral Examination of American Board of Obstetrics and Gynecology

If you passed the written examination on or before June 30, 1979, you may be eligible to apply for the oral examination to be held in 1981. The appropriate forms and current bulletin may be obtained by writing to the Office of the Secretary, at the address shown below, in November-December of 1980. The deadline date for the receipt of applications in the office of the secretary is February 28, 1981.

To be ruled eligible, candidates must have unrestricted hospital privileges with independent unsupervised patient care. Candidates who are subspecialty Fellows are required to have one year of independent practice with unrestricted hospital privileges following the Fellowship. All candidates will be required to submit a list of all patients discharged from hospitals for a one-year span.

James A. Merrill, M.D., Secretary-Treasurer, The

American Board of Obstetrics and Gynecology, Inc., 711 Stanton Young Boulevard, Oklahoma City, Oklahoma 73104.

Twenty-second Annual Obstetrics and Gynecology Update

The Department of Obstetrics and Gynecology, University of Utah College of Medicine, will present a clinically oriented postgraduate course which will be held at the Holiday Inn, Park City, Utah, February 21-24, 1981. Registrants will be updated in a variety of topics in obstetrics and gynecology. Presenting along with University of Utah faculty will be William Spellacy, M.D., C. Don Christian, M.D., and John Hobbins, M.D. This course has been approved for 15 cognates, Formal Learning, by The American College of Obstetricians and Gynecologists. The University of Utah certifies that this Continuing Medical Education activity meets the criteria for 15 hours in Category I of the American Medical Association.

For further information contact: David H. Engleberg, Department of Obstetrics and Gynecology, University of Utah College of Medicine, 50 North Medical Dr., Salt Lake City, Utah 84132.

Gynecologic Oncology Program

The Department of Obstetrics and Gynecology, University of Utah College of Medicine, will present a clinically oriented postgraduate course which will be held at the Holiday Inn, Park City, Utah, February 25-28, 1981. Registrants will be updated in the subspecialty of gynecologic oncology. Presenting along with University of Utah faculty will be some of the foremost gynecologic oncologists in the United States today. This course has been approved for 13 cognates, Formal Learning, by The American College of Obstetricians and Gynecologists. The University of Utah certifies that this Continuing Medical Education activity meets the criteria for 15 hours in Category I of the American Medical Association.

For further information contact: David H. Engleberg, Department of Obstetrics and Gynecology, University of Utah College of Medicine, 50 North Medical Dr., Salt Lake City, Utah 84132.

Fourth European Congress on Ultrasonics in Medicine

The Fourth European Congress on Ultrasonics in Medicine will be held May 17-24, 1981, at the Hotel Libertas in Dubrovnik, Yugoslavia. It is sponsored by the European Federation of Societies for Ultrasound in Medicine and Biology.

For information write: Yugoslav Association of Societies for Ultrasound in Medicine and Biology, Braće Kavurića 17, 41000 Zagreb, Yugoslavia.

Endocrinology of Reproduction

The Department of Obstetrics and Gynecology, Cornell University Medical College, in collaboration with the American College of Obstetricians and Gynecologists, will present a comprehensive postgraduate course, "Endocrinology of Reproduction," at the Cornell University Medical College in New York, New York, December 5-7, 1980.

For further information contact: Fritz Fuchs, M.D., Program Director, 530 East 70th St., New York, New York 10021.

Advanced Educational Seminar: Diagnostic Ultrasound

An Advanced Educational Seminar on Diagnostic Ultrasound will be held January 26-30, 1981. It is sponsored by the Health Sciences Centre, Winnipeg, Manitoba, Canada.

For further information contact: Advanced Educational Program, Department of Diagnostic Ultrasound, Health Sciences Centre, 700 William Ave., Winnipeg, Manitoba, Canada R3E 0Z3.

Update in Obstetrics and Gynecology: 1981

Physicians are cordially invited to attend "Update in Obstetrics and Gynecology: 1981," to be held at the Caribe Hilton in San Juan, Puerto Rico, March 2-6, 1981, sponsored by the Departments of Gynecology and Obstetrics, The University of Puerto Rico and The Johns Hopkins University School of Medicine.

For further information contact: Program Coordinator, Continuing Education, Turner Auditorium Room 19, 720 Rutland Ave., Baltimore, Maryland 21205.

Fourth National Conference on Rural Primary Care

The National Rural Primary Care Association is pleased to announce that they will be sponsoring a call for papers for the Fourth National Conference on Rural Primary Care. The Conference will be held on March 7-12, 1981, in Denver, Colorado.

For information write: Search Committee, NRPCA, Box 1211, Waterville, Maine 04901, Attn: Vicki Coates.

Microsurgery Course

The Johns Hopkins Medical Institutions is offering a one-week Microsurgery Course to be held continuously throughout the year, scheduled from 8:00 AM to 5:00 PM daily.

For further information contact: Program Coordinator, Continuing Education, Turner 22, 720 Rutland Ave., Baltimore, Maryland 21205.

Winter Conference on ultrasound, computed tomography, and nuclear medicine

The "Winter Conference" on ultrasound, computed tomography and nuclear medicine will be held February 1-8, 1981, at the Sahara Tahoe Hotel, Lake Tahoe (Stateline), Nevada.

For information write: American College of Medical Imaging, P. O. Box 27188, Los Angeles, California 90027.

Pan-American Conference on Fertility and Sterility

The Pan-American Conference on Fertility and Sterility will be held February 12-20, 1981, at the Mullet Bay Beach Hotel, St. Maarten, Netherlands, Antilles.

A Pre-Conference Postgraduate Course will be presented February 12, 1981.

For scientific details and information contact: U. S. International Foundation for Studies in Reproduction, Inc., 112-44 69th Ave., Forest Hills, New York 11375.

For information on travel and accommodations contact: Medical Congress Coordinators, 1212 Avenue of the Americas, New York, New York 10036.

University of Florida—*Disney World 1981 Update in OB/GYN*

The University of Florida—Disney World 1981 Update in OB/GYN will be held February 9-11, 1981, at the Marriott Inn, Orlando, Florida.

For information contact: Frank C. Riggall, M.D., Box J-294, Shands Teaching Hospital, Gainesville, Florida 32610.

Postgraduate course in Gynecologic and Obstetric Pathology

The Departments of Pathology, Massachusetts General Hospital and Boston Hospital for Women, are sponsoring a postgraduate course in Gynecologic and Obstetric Pathology, April 13-17, 1981.

For further information, please contact: Department of Continuing Education, Harvard Medical School, 25 Shattuck St., Boston, Massachusetts 02115.

Tenth Meeting of the International Study Group for Steroid Hormones

The Tenth Meeting of the International Study Group for Steroid Hormones will be held in Rome, Italy December 2-4, 1981. The topic will be "Hormone Factors in Fertility, Infertility, and Contraception." The meeting is sponsored by Organon International BV, Radim.

For information write: Prof. C. Conti, Clinica Medica v. Universita di Roma, Policlinico Umberto I, 00100 Rome, Italy.

Second Annual Patient Education Research Seminar

The Second Annual Patient Education Research Seminar will be held December 6-7, 1980, at the University of California, San Francisco, Cole Hall.

For registration information contact: University of California, San Francisco, Continuing Education in Health Sciences, 1308 3rd Ave., San Francisco, California 94143. For program information contact: University of California, San Francisco, Continuing Education in Health Sciences, 24 Kirkham Ave., San Francisco, California 94143.

BOOKS

Books received

Epidemiology of Anencephalus and Spina Bifida. J. Mark Elwood and J. Harold Elwood. 413 pages, illustrated. New York, 1980, Oxford University Press. $69.50.

Frozen Human Semen. A Royal College of Obstetricians and Gynaecologists Workshop on Cryobiology of Human Semen and Its Role in Artificial Insemination by Donor, March 22 and 23, 1979. Edited by D. W. Richardson, D. Joyce, and E. M. Symonds. 272 pages. Boston, 1980, Kluwer Boston, Inc. $49.50.

Hypertension in Pregnancy Concepts and Management. Norman F. Gant, Jr., and Richard J. Worley. 211 pages, illustrated, New York, 1980, Appleton-Century-Crofts. No price listed.

Management of High Risk Pregnancy. Edited by John T. Queenan. 705 pages, illustrated. Oradell, New Jersey, 1980, Medical Economics Books. No price listed.

Managing Sexual Dysfunction. A Basic Guide. John F. O'Connor. 156 pages, illustrated. Oradell, New Jersey, 1980, Medical Economics Company. $9.95. (soft cover).

Microsurgery in Female Infertility. Edited by P. G. Crosignani and B. L. Rubin. 134 pages, illustrated. New York, 1980, Grune & Stratton. $21.00 (soft cover).

Oxford Reviews of Reproductive Biology. Vol. 1, 1979. Edited by C. A. Finn. 485 pages. New York, 1979, Oxford University Press. $67.50.

The Safety of Fertility Control. Vol. 1. Focus on Women. Edited by Louis G. Keith, Deryck R. Kent, Gary S. Berger, and Janelle R. Brittain. 360 pages, illustrated. New York, 1980, Springer Publishing Company. $23.50.

Sports Medicine for the Athletic Female. Edited by Christine E. Haycock. 412 pages, illustrated. Florence, Kentucky, 1980, Medical Economics Books. $27.50.

Surgical Geriatric Gynecology. Contributions to Gynecology and Obstetrics. Vol. 7. V. Jaluvka. 173 pages, illustrated. White Plains, New York, 1980, Albert J. Phiebig, Inc. $53.50 (soft cover).

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New! ARTIFICIAL INSEMINATION WITH HUSBAND SPERM

By **Wilfred J. Finegold,** *Pittsburgh, Pennsylvania.* Designed for the infertile couple, this book explains the practice of artificial insemination with the husband's sperm (AIH). The author describes the development of artificial insemination — generally done, in the past, with donor sperm — and its recent successes using the sperm of the husband.

The text begins by familiarizing the reader with the nomenclature involved in artificial insemination and the history of both AIH and artificial insemination with donor sperm (AID). Background information on the relation of artificial insemination to animal husbandry, the physiology of reproduction, and the procedures used in AID follows. Ovulation, the differences between AIH and AID, and the burgeoning interest in and use of AIH also are discussed.

Indications for and methods used in the AIH process are fully described. The author explains the usefulness of AIH in cases involving retrograde ejaculation, split ejaculate, oligospermia, immunological factors, and hostile cervical mucus. He reviews psychological and religious considerations as well, and he includes data on such topics as test-tube babies, sex preferences, and surrogate motherhood. The text closes with chapters on sperm freezing, the separation and filtration of sperm in relation to AIH, and the use of donor seminal plasma in AIH.

Because the text is aimed at the patient-couple rather than the expert gynecologist, the author presents the material in a straightforward, nontechnical manner. Thus, it will be easily understood by the layperson, but general medical practitioners and specialists in areas other than obstetrics/gynecology will find the book accurate, up-to-date and useful in understanding the rationale for and techniques of artificial insemination with husband sperm. *'80, 96 pp., $14.75*

New! **THE DEVELOPMENT OF THE BRAIN: Biological and Functional Perspectives** *by* **Stanislav Reinis** *and* **Jerome M. Goldman,** *both of Univ. of Waterloo, Waterloo, Ontario, Canada. '80, 416 pp., 192 il., 17 tables, $29.75*

PRENATAL PATHOLOGY: Fetal Medicine *by* **T. V. N. Persaud,** *Univ., of Manitoba, Winnipeg, Manitoba, Canada, in collaboration with* **M. Ray** *and* **H. Rigatto.** *Foreword by* **T. M. Roulston.** *'79, 212 pp., 76 il., 31 tables, $25.25*

NUTRITIONAL FACTORS IN GENERAL MEDICINE: Effects of Stress and Distorted Diets *by* **Mark D. Altschule,** *Harvard Medical School, Boston. '78, 200 pp., $11.75*

GENETICS ISSUES IN PUBLIC HEALTH AND MEDICINE *edited by* **Bernice H. Cohen, Abraham M. Lilienfeld** *and* **P. C. Huang,** *all of Johns Hopkins Univ. School of Hygiene and Public Health, Baltimore, Maryland. (28 Contributors) '78, 512 pp., 44 il., 42 tables, $34.50*

PATHOPHYSIOLOGY OF THE KIDNEY *edited by* **Neil A. Kurtzman,** *Univ. of Illinois, Chicago, and* **Manuel Martinez-Maldonado,** *Univ. of Puerto Rico, San Juan. (40 Contributors) '77, 1104 pp. (6 3/4 x 9 3/4), 266 il., 64 tables, $95.00*

THE HUMAN FETAL AND NEONATAL CIRCULATION: Function and Structure *by* **S. Zoe Walsh,** *Karolinska Hospital and Institute, Stockholm, Sweden;* **W. W. Meyer,** *Univ. of Mainz, Mainz, Federal Republic of Germany; and* **John Lind,** *Karolinska Hospital and Institute, Stockholm, Sweden. '74, 368 pp. (7 x 10), 275 il. (68 in color), 22 tables, $19.00*

SEXUALLY TRANSMITTED DISEASES *compiled and edited by* **Leslie Nicholas,** *Hahnemann Medical College and Hospital, Philadelphia. Foreword by* **Herman Beerman.** *(22 Contributors) '73, 264 pp., 42 il., 29 tables, $18.50*

THE PRENATAL DIAGNOSIS OF HEREDITARY DISORDERS *by* **Aubrey Milunsky,** *Harvard Univ., Boston. Foreword by* **John W. Littlefield.** *'73, 276 pp., 18 il. (2 in color), 26 tables, $15.25*

MATERNAL NUTRITION AND CHILD HEALTH *by* **Douglas R. Shanklin,** *Univ. of Chicago, and* **Jay Hodin,** *Society for the Protection of the Unborn Through Nutrition, both of Chicago. Foreword by* **S. L. Manocha.** *'79, 224 pp., 9 il., 68 tables, $16.25*

New! **TEENAGE PREGNANCY: Research Related to Clients and Services** *by* **Jean E. Bedger,** *United Way of Metropolitan Chicago, Chicago. (3 Contributors) '80, 224 pp., 43 tables, $16.75*

COLPOSCOPY: A Scientific and Practical Approach to the Cervix and Vagina in Health and Disease (2nd Ed.) *by* **Malcolm Coppleson,** *King George V Memorial Hospital, Sydney, Australia;* **Ellis Pixley,** *King Edward Memorial Hospital, Perth, Australia; and* **Bevan Reid,** *Univ. of Sydney, Sydney, Australia. (5 Contributors) '78, 512 pp. (6 3/4 x 9 3/4), 306 il. (1 in color), 11 tables, $40.25*

GENITOURINARY PROBLEMS IN WOMEN *by* **Jack R. Robertson,** *Univ. of Southern California Medical Center, Los Angeles. Foreword by* **Richard W. TeLinde.** *'78, 168 pp., 105 il. (32 in color), 2 tables, $20.25*

CHRONOGENETICS: The Inheritance of Biological Time *by* **Luigi Gedda** *and* **Gianni Brenci,** *both of Gregor Mendel Institute of Medical Genetics and Gemellology, Rome, Italy. '78, 232 pp., 65 il., 27 tables, $24.00*

RAPE VICTIMOLOGY *edited by* **LeRoy G. Schultz,** *West Virginia Univ., Morgantown. (19 Contributors) '75, 424 pp., 13 tables, $35.50*

THE PLACENTA: Biological and Clinical Aspects *edited by* **Kamran S. Moghissi** *and* **E. S. E. Hafez,** *both of Wayne State Univ., Detroit, Michigan. (29 Contributors) '74, 412 pp., 162 il., 30 tables, $37.50*

BIRTH DEFECTS AND FETAL DEVELOPMENT: Endocrine and Metabolic Factors *edited by* **Kamran S. Moghissi,** *Wayne State Univ., Detroit, Michigan. (22 Contributors) '74; 352 pp., 94 il. (1 in color), 45 tables, $31.00*

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Contact:
Arthur Robinson, M.D.
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For further information contact:
R. M. Pitkin, M.D., Professor and Head
Department of Obstetrics and Gynecology
University of Iowa Hospitals
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A. Total no. copies printed *(net press run)*	21,220	20,974
B. Paid circulation		
1. Sales through dealers and carriers, street vendors and counter sales	None	None
2. Mail subscriptions	20,355	19,722
C. Total paid circulation	20,355	19,722
D. Free distribution by mail, carrier or other means, samples, complimentary, and other free copies	669	431
E. Total distribution *(Sum of C and D)*	21,024	20,153
F. Copies not distributed		
1. Office use, left over, unaccounted, spoiled after printing	196	821
2. Returns from news agents	None	None
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11. I certify that the statements made by me above are correct and complete.

A. JEROME FREELAND, *Vice President and Journal Publisher*

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volume 138, *number* 6

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American Journal of OBSTETRICS AND GYNECOLOGY

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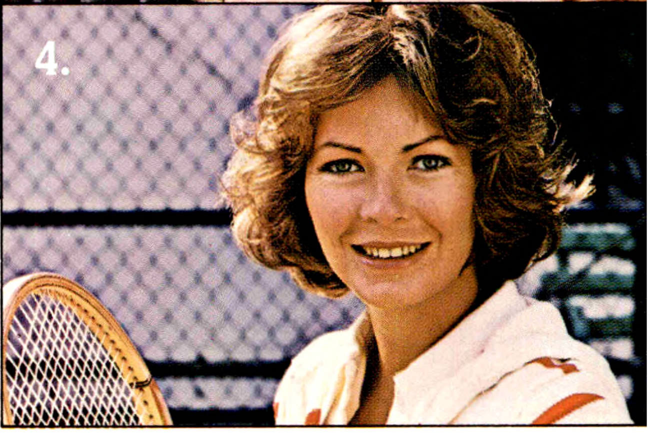

1. She wants convenience.
2. She wants nonhormonal contraception.
3. She smokes.
4. She is over 35.

Cu-7® / Tatum-T™
(intrauterine copper contraceptives)

Answer their contraceptive priorities...

1. For the contemporary woman who wants freedom from rigid schedules, the copper IUD is a logical choice because of its inherent convenience.

2. For the new mother who wants to space her family...the copper IUD has no effect on lactation. Its efficacy rate is almost as high as that of the Pill. Therefore, the postpartum period may be an especially opportune time to insert a Cu-7 or Tatum-T, providing involution has occurred.

3. For the woman—of any age—who smokes, the copper IUD produces no problems in association with smoking.

4. For the woman who has passed the Pill years, but who still wants highly effective and reversible contraception, the copper IUDs are a logical choice.

Intrauterine contraception is not appropriate for all women. See complete prescribing information prior to use.
Please see next page for a brief summary of product information.

Cu-7®/ Tatum-T™

(intrauterine copper contraceptives)

Description: The Cu-7 has coiled around the vertical limb 89 mg. of copper wire providing approximately 200 mm² of exposed copper surface area. The Tatum-T has coiled around the vertical limb 120 mg. of copper wire providing approximately 210 mm² of exposed copper surface area.

Contraindications: Not to be inserted when any of the following conditions exist: pregnancy or suspicion of pregnancy; abnormalities of the uterus resulting in distortion of the uterine cavity; acute pelvic inflammatory disease or history of repeated pelvic inflammatory disease; postpartum endometritis or infected abortion in the past three months; known or suspected uterine or cervical disease such as hyperplasia or carcinoma including unresolved, abnormal "Pap" smear; vaginal bleeding of unknown etiology; untreated acute cervicitis until infection is controlled; diagnosed Wilson's disease; known or suspected allergy to copper; previous ectopic pregnancy; significant anemia; and valvular heart disease, leukemia, or use of chronic corticosteroid therapy.

Warnings

1. Pregnancy: (a) Long-term effects. The long-term effects on the offspring of the presence of copper in the uterus when pregnancy occurs are unknown.

(b) Septic abortion. Reports have indicated an increased incidence of septic abortion associated in some instances with septicemia, septic shock and death in patients becoming pregnant with an IUD in place. Most of these reports have been associated with the mid-trimester of pregnancy. In some cases, the initial symptoms have been insidious and not easily recognized. If pregnancy should occur with a Cu-7 or Tatum-T in situ, the IUD should be removed if the thread is visible or, if removal proves to be or would be difficult, interruption of the pregnancy should be considered and offered to the patient as an option, bearing in mind that the risks associated with an elective abortion increase with gestational age.

(c) Continuation of pregnancy. If the patient chooses to continue the pregnancy and the Cu-7 or Tatum-T remains in situ, she must be warned of increased risk of spontaneous abortion and increased risk of sepsis, including death. The patient must be closely observed and advised to report immediately all abnormal symptoms, such as flu-like syndrome, fever, abdominal cramping and pain, bleeding, or vaginal discharge, because generalized symptoms of septicemia may be insidious.

2. Ectopic pregnancy: (a) Pregnancy which occurs with an IUD in situ is more likely to be ectopic than pregnancy occurring without an IUD. Therefore, patients who become pregnant while using a Cu-7 or Tatum-T should be carefully evaluated for the possibility of ectopic pregnancy.

(b) Special attention should be directed to patients with delayed menses, slight metrorrhagia and/or unilateral pelvic pain, and to those patients who wish to interrupt a pregnancy occurring in the presence of a Cu-7 or Tatum-T, to determine whether ectopic pregnancy has occurred.

3. Pelvic infection: An increased risk of pelvic inflammatory disease associated with the use of IUDs has been reported. While unconfirmed, this risk appears to be greatest for young women who are nulliparous and/or who have multiple sexual partners. Salpingitis can result in tubal damage and occlusion, thereby threatening future fertility. Therefore, it is recommended that patients be taught to look for symptoms of pelvic inflammatory disease. The decision to use an IUD in a particular case must be made by the physician and patient with consideration of a possible deleterious effect on future fertility.

Pelvic infection may occur with a Cu-7 or Tatum-T in situ and at times result in the development of tubo-ovarian abscesses or general peritonitis. The symptoms of pelvic infection include: new development of menstrual disorders (prolonged or heavy bleeding), abnormal vaginal discharge, abdominal or pelvic pain, dyspareunia, fever. The symptoms are especially significant if they occur following the first few cycles after insertion. Appropriate aerobic and anaerobic bacteriologic studies should be done and antibiotic therapy initiated promptly. If the infection does not show marked clinical improvement within 24 to 48 hours, the Cu-7 or Tatum-T should be removed and continuing treatment reassessed on the basis of results of culture and sensitivity tests.

4. Embedment: Partial penetration or lodging of the Cu-7 or Tatum-T in the endometrium or myometrium can result in difficult removal. This may occur more frequently in smaller uteri. *See removal instructions.*

5. Perforation: Partial or total perforation of the uterine wall or cervix may occur, usually during insertions into patients sooner than two months after abortion or delivery, or in uterine cavities too small. The possibility of perforation must be kept in mind during insertion and at the time of any subsequent examination. If perforation occurs, laparotomy or laparoscopy should be performed as soon as medically feasible and the Cu-7 or Tatum-T removed. Abdominal adhesions, intestinal penetration, intestinal obstruction, and local inflammatory reaction with abscess formation and erosion of adjacent viscera may result if the Cu-7 or Tatum-T is left in the peritoneal cavity.

6. Medical diathermy: The use of medical diathermy (short-wave and microwave) in a patient with a metal-containing IUD may cause heat injury to surrounding tissue. Therefore, medical diathermy to the abdominal and sacral areas should not be used on patients using a Cu-7 or Tatum-T.

7. Effects of copper: Additional amounts of copper available to the body from the Cu-7 or Tatum-T may precipitate symptoms in women with undiagnosed Wilson's disease. The incidence of Wilson's disease is 1 in 200,000.

Precautions

1. Patient counseling. Prior to insertion the physician, nurse, or other trained health professional must provide the patient with the Patient Brochure. The patient should be given the opportunity to read the brochure and discuss fully any questions she may have concerning the Cu-7 or Tatum-T as well as other methods of contraception.

2. Patient evaluation and clinical considerations. (a) A complete medical history should be obtained to determine conditions that might influence the selection of an IUD. A physical examination should include a pelvic examination, "Pap" smear, gonorrhea culture and, if indicated, appropriate tests for other forms of venereal disease. The physician should determine that the patient is not pregnant.

(b) The uterus should be carefully sounded prior to insertion to determine the degree of patency of the endocervical canal and internal os, and the direction and depth of the uterine cavity. Exercise care to avoid perforation with the sound. DO NOT USE THE INSERTION INSTRUMENT AS A SOUND. In occasional cases, severe cervical stenosis may be encountered. Do not use excessive force to overcome this resistance.

(c) The uterus usually sounds to a depth of 6 to 8 cm. Insertion into a uterine cavity measuring less than 6.5 cm. by sounding may increase the incidence of pain, bleeding, partial or complete expulsion, perforation, and possibly pregnancy.

(d) To reduce the possibility of insertion in the presence of existing undetermined pregnancy, the optimal time for insertion is the latter part of the menstrual flow or one or two days thereafter. The Cu-7 or Tatum-T should not be inserted post partum or post abortion until involution of the uterus is complete. The incidence of perforation and expulsion is greater if involution is not complete.

It is, however, necessary to place the Cu-7 or Tatum-T as high as possible within the uterine cavity to help avoid partial or complete expulsion that could result in pregnancy.

Physicians are cautioned that it is imperative for them to become thoroughly familiar with the instructions for use before attempting placement of the Cu-7 or Tatum-T.

(e) IUDs should be used with caution in those patients who have anemia or a history of menorrhagia or hypermenorrhea. Patients experiencing menorrhagia and/or metrorrhagia following IUD insertion may be at risk for the development of hypochromic microcytic anemia. Also, IUDs should be used with caution in patients receiving anticoagulants or having a coagulopathy.

(f) Syncope, bradycardia or other neurovascular episodes may occur during insertion or removal of IUDs, especially in patients with a previous disposition to these conditions.

(g) Patients with valvular or congenital heart disease are more prone to develop subacute bacterial endocarditis than patients who do not have such disease. Use of an IUD in these patients may represent a potential source of septic emboli.

(h) Use of an IUD in patients with acute cervicitis should be postponed until proper treatment has cleared up the infection.

(i) Since the Cu-7 or Tatum-T may be partially or completely expelled, patients should be reexamined and evaluated shortly after the first postinsertion menses, but definitely within three months after insertion. Thereafter annual examination with appropriate medical and laboratory evaluation should be carried out. The Cu-7 or Tatum-T should be replaced every three years.

(j) The patient should be told that some bleeding or cramping may occur during the first few weeks after insertion, but if these symptoms continue or are severe she should report them to her physician. She should be instructed on how to check after each menstrual period to make certain that the thread still protrudes from the cervix and cautioned that there is no contraceptive protection if the Cu-7 or Tatum-T has been expelled. She should also be cautioned not to dislodge the Cu-7 or Tatum-T by pulling on the thread. If a partial expulsion occurs, removal is indicated and a new Cu-7 or Tatum-T may be inserted. The patient should be told to return within three years for removal of the Cu-7 or Tatum-T and for replacement if desired.

(k) A copper-induced urticarial allergic skin reaction may develop in women using a copper-containing IUD. If symptoms of such an allergic response occur, the patient should be instructed to tell the consulting physician that a copper-containing device is being used.

(l) The Cu-7 or Tatum-T should be removed for the following medical reasons: menorrhagia and/or metrorrhagia producing significant anemia; uncontrolled pelvic infection; intractable pain often aggravated by intercourse, dyspareunia; pregnancy, if the thread is visible; endometrial or cervical malignancy; uterine or cervical perforation; or any indication of partial expulsion.

(m) If the retrieval thread cannot be seen it may have retracted into the uterus or have been broken off, or the Cu-7 or Tatum-T may have been expelled. Localization usually can be made by feeling with a probe; if not, x-ray or sonography can be used. When the physician elects to recover a Cu-7 or Tatum-T with the thread not visible, the removal instructions should be considered.

(n) If any patient with a Cu-7 or Tatum-T suddenly develops overt clinical hepatitis or abnormal liver function tests, appropriate diagnostic procedures should be initiated.

Adverse Reactions: Perforations of uterus and cervix have occurred. Perforation into the abdomen has been followed by abdominal adhesions, intestinal penetration, intestinal obstruction, local inflammatory reaction with abscess formation and erosion of adjacent viscera. Pregnancy has occurred with the Cu-7 or Tatum-T in situ and when either has been partially or completely expelled.

The incidence of spontaneous abortion, when conception occurs with intrauterine devices in situ, appears to be increased over that in unprotected women. Insertion cramping, usually of no more than a few seconds' duration, may occur; however, some women may experience residual cramping for several hours or even days. Intermenstrual spotting or bleeding or prolonged or increased menstrual flow may occur.

Pelvic infection including salpingitis with tubal damage or occlusion has been reported. This may result in future infertility. Complete or partial expulsion of the Cu-7 or Tatum-T may sometimes occur, particularly in those patients with uteri measuring less than 6.5 cm. by sounding. Urticarial allergic skin reaction may occur. The following complaints have also been reported with IUDs although their relation to the Cu-7 or Tatum-T has not been established: amenorrhea or delayed menses, backaches, cervical erosion, cystic masses in the pelvis, vaginitis, leg pain or soreness, weight loss or gain, nervousness, dyspareunia, cystitis, endometritis, septic abortion, septicemia, leukorrhea, ectopic pregnancy, difficult removal, uterine embedment, anemia, pain, neurovascular episodes including bradycardia and syncope secondary to insertion, dysmenorrhea, and fragmentation of the IUD.

Clinical Efficacy: In clinical trials, use effectiveness was determined as follows for parous and nulliparous women, as tabulated by the life table method. (Rates are expressed as cumulative events per 100 women through 12, 24, and 36 months of use.)

Cu-7 experience encompasses 387,131 woman-months of use, including 12 months for 11,517 women, 24 months for 7,895, and 36 months for 3,661.

Tatum-T experience encompasses 236,060 woman-months of use in the United States and Canada, including 12 months for 8,232 women, 24 months for 4,247, and 36 months for 1,408.

Cumulative pregnancy rates were:

	12 Months		24 Months		36 Months*	
	Parous	Nulliparous	Parous	Nulliparous	Parous	Nulliparous
Cu-7	1.9	1.7	2.9	2.6	3.4	3.4
Tatum-T	3.0	2.1	4.9	4.5	6.0	5.8

Please see complete product labeling for additional information, including insertion and removal instructions.

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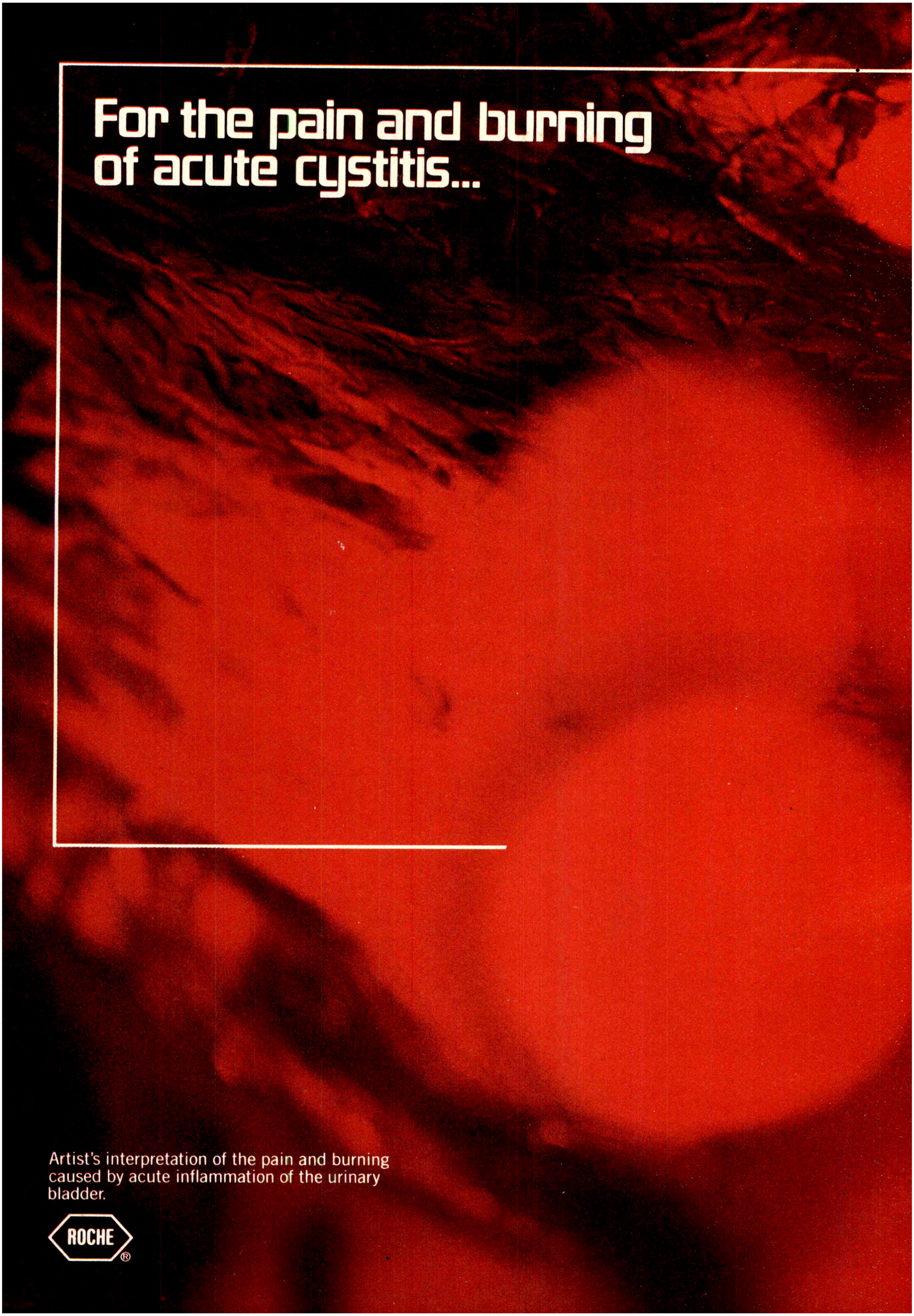
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Fast pain relief plus effective antibacterial action

In nearly 9 out of 10 patients, Azo Gantanol reduced the pain and/or burning of acute cystitis within 24 hours.*

A controlled, multicenter study assessed the efficacy of Azo Gantanol in relieving the pain and/or burning associated with acute urinary tract infection. Patients included in the study had at least 100,000 colonies per ml of a sulfonamide-sensitive organism, usually *E. coli.* In 87% of patients (303 of 349) with initial symptoms rated "moderate to severe," therapy with Azo Gantanol resulted in improvement within 24 hours.†

Azo Gantanol combines fast symptomatic relief with effective antibacterial action. The analgesic phenazopyridine ("Azo") rapidly relieves pain and burning, while the antibacterial sulfamethoxazole ("Gantanol") achieves therapeutic blood and urine levels from 2 to 3 hours after the initial 2-Gm dose.

Patients should be cautioned to maintain adequate fluid intake. Azo Gantanol and Gantanol (sulfamethoxazole/Roche) are contraindicated during pregnancy and the nursing period. During therapy, perform frequent CBC's and urinalyses with careful microscopic examination.

† Data on file, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

4 tablets initially followed by 2 tablets B.I.D.

Azo Gantanol®

Each tablet contains 0.5 Gm sulfamethoxazole and 100 mg phenazopyridine HCl.

for the pain— for the pathogens

*Azo Gantanol recommended for relief of pain, followed by Gantanol (sulfamethoxazole/Roche) for remaining course of therapy.

Please see brief summary of complete product information on following page.

Azo Gantanol®

Each tablet contains 0.5 Gm sulfamethoxazole and 100 mg phenazopyridine HCl.

Before prescribing, please consult complete product information, a summary of which follows:

INDICATIONS: In adults, urinary tract infections complicated by pain (primarily pyelonephritis, pyelitis and cystitis) due to susceptible organisms (usually *E. coli, Klebsiella-Aerobacter, Staphylococcus aureus, Proteus mirabilis*, and, less frequently, *Proteus vulgaris*) in the absence of obstructive uropathy or foreign bodies.

Note: Carefully coordinate *in vitro* sulfonamide sensitivity tests with bacteriologic and clinical response; add aminobenzoic acid to follow-up culture media. The increasing frequency of resistant organisms limits the usefulness of antibacterials including sulfonamides. Measure sulfonamide blood levels as variations may occur; 20 mg/100 ml should be maximum total level.

CONTRAINDICATIONS: Children below age 12; sulfonamide hypersensitivity; pregnancy at term and during nursing period; because Azo Gantanol contains phenazopyridine hydrochloride it is contraindicated in glomerulonephritis, severe hepatitis, uremia, and pyelonephritis of pregnancy with G.I. disturbances.

WARNINGS: Safety during pregnancy not established. Deaths from hypersensitivity reactions, agranulocytosis, aplastic anemia and other blood dyscrasias have been reported and early clinical signs (sore throat, fever, pallor, purpura or jaundice) may indicate serious blood disorders. Frequent CBC and urinalysis with microscopic examination are recommended during sulfonamide therapy.

PRECAUTIONS: Use cautiously in patients with impaired renal or hepatic function, severe allergy, bronchial asthma; in glucose-6-phosphate dehydrogenase-deficient individuals in whom dose-related hemolysis may occur. Maintain adequate fluid intake to prevent crystalluria and stone formation.

ADVERSE REACTIONS: *Blood dyscrasias* (agranulocytosis, aplastic anemia, thrombocytopenia, leukopenia, hemolytic anemia, purpura, hypoprothrombinemia and methemoglobinemia); *allergic reactions* (erythema multiforme, Stevens-Johnson syndrome, skin eruptions, epidermal necrolysis, urticaria, serum sickness, pruritus, exfoliative dermatitis, anaphylactoid reactions, periorbital edema, conjunctival and scleral injection, photosensitization, arthralgia and allergic myocarditis); *G.I. reactions* (nausea, emesis, abdominal pains, hepatitis, diarrhea, anorexia, pancreatitis and stomatitis); *CNS reactions* (headache, peripheral neuritis, mental depression, convulsions, ataxia, hallucinations, tinnitus, vertigo and insomnia); *miscellaneous reactions* (drug fever, chills, toxic nephrosis with oliguria and anuria, periarteritis nodosa and L. E. phenomenon). Due to certain chemical similarities with some goitrogens, diuretics (acetazolamide, thiazides) and oral hypoglycemic agents, sulfonamides have caused rare instances of goiter production, diuresis and hypoglycemia. Cross-sensitivity with these agents may exist.

DOSAGE: Azo Gantanol is intended for the acute, painful phase of urinary tract infections. *Usual adult dosage:* 2 Gm (4 tabs) initially, then 1 Gm (2 tabs) B.I.D. for up to 3 days. If pain persists, causes other than infection should be sought. After relief of pain has been obtained, continued treatment with Gantanol (sulfamethoxazole/Roche) may be considered.

NOTE: Patients should be told that the orange-red dye (phenazopyridine HCl) will color the urine.

SUPPLIED: Tablets, red, film-coated, each containing 0.5 Gm sulfamethoxazole and 100 mg phenazopyridine HCl—bottles of 100 and 500.

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Vol. 138, No. 6, November 15, 1980. The American Journal of Obstetrics and Gynecology is published semimonthly by The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63141. POSTMASTER: Send address changes to above address.

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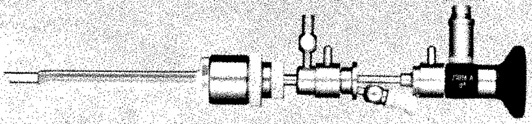

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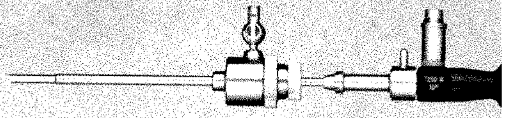

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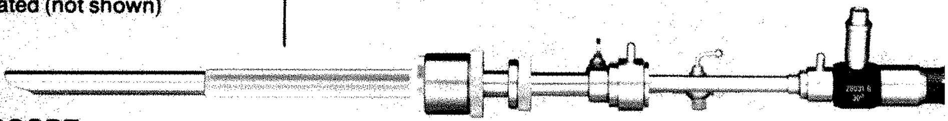

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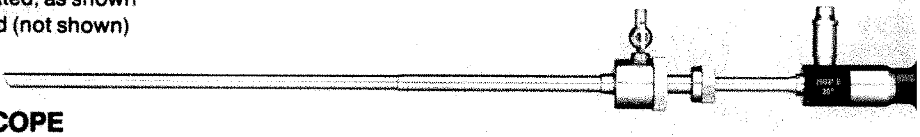

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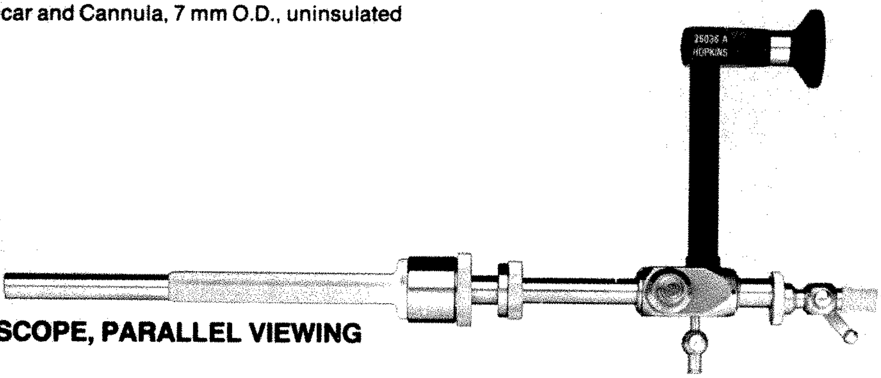

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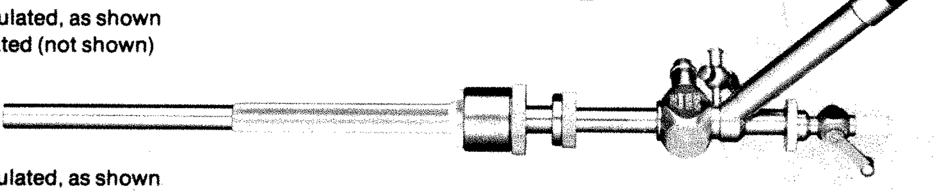

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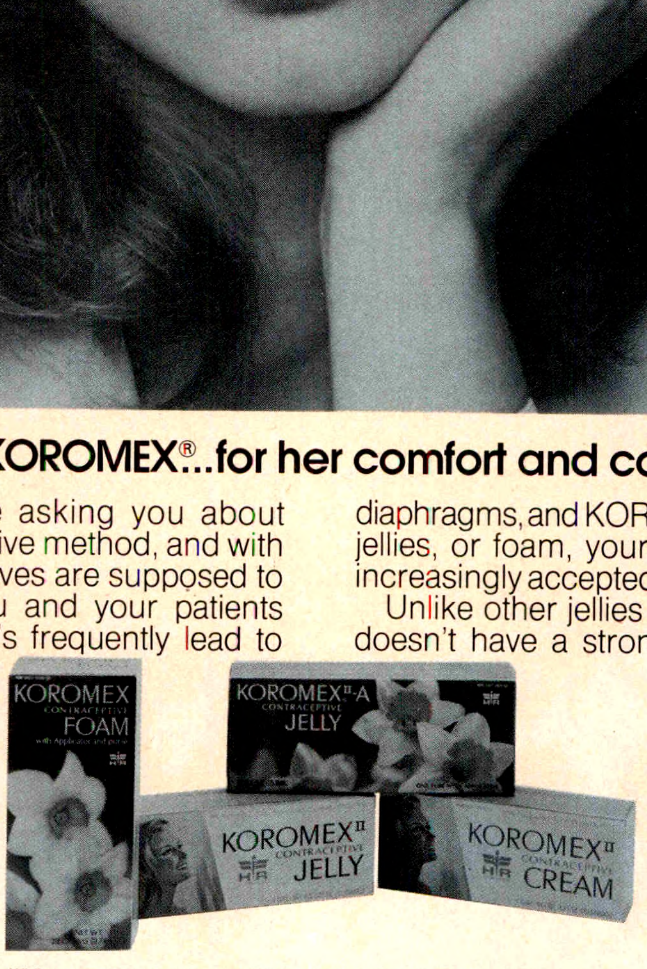
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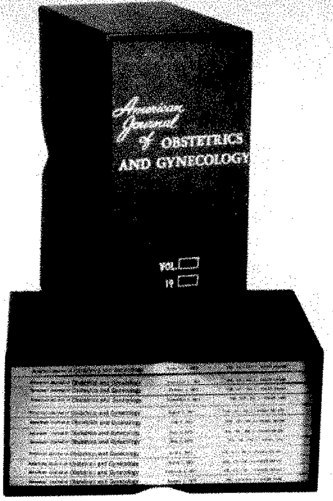

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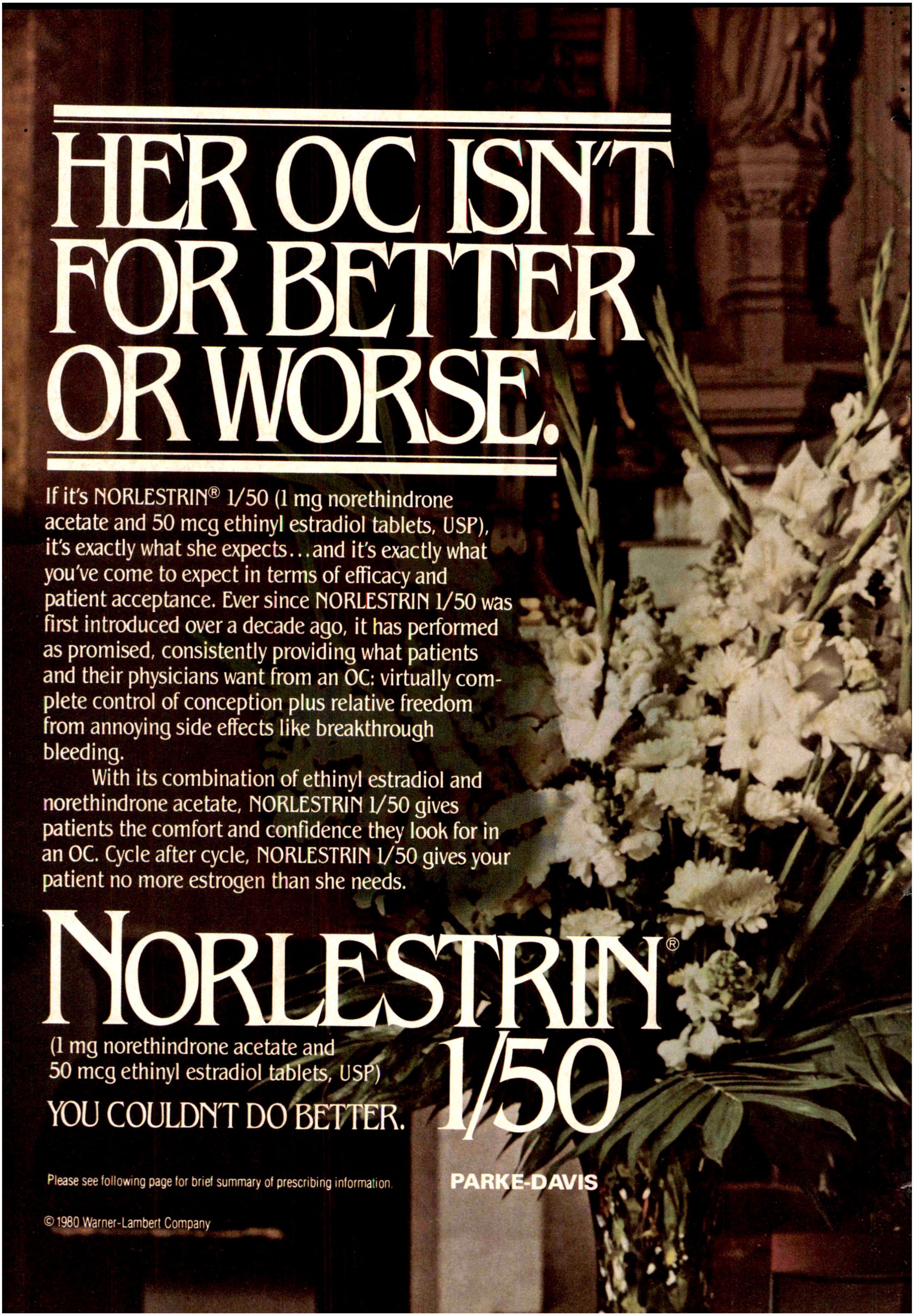

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CONTRAINDICATIONS

1. Thrombophlebitis or thromboembolic disorders
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders
3. Cerebral vascular or coronary artery disease
4. Known or suspected carcinoma of the breast
5. Known or suspected estrogen-dependent neoplasia
6. Undiagnosed abnormal genital bleeding
7. Known or suspected pregnancy (See WARNING No. 5.)

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, and hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well-established. Studies have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic.

Cerebrovascular disorders: In a collaborative study in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 4.0 to 9.5 times greater.

Myocardial infarction: An increased risk of myocardial infarction associated with oral contraceptives has been reported confirming a previously suspected association. These studies found that the greater the number of underlying risk factors (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) for coronary artery disease, the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor.

It has been estimated that users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are smokers have about a fivefold increased risk of fatal infarction compared to users who do not smoke, but about a tenfold to twelvefold increased risk compared to nonusers who do not smoke. The amount of smoking is also an important factor.

Risk of dose: In an analysis of data, British investigators conluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; however, the quantity of estrogen may not be the sole factor involved.

Estimate of excess mortality from circulatory diseases: The risk of diseases of the circulatory system is concentrated in older women, in those with a long duration of use, and in cigarette smokers.

A study of available data from a variety of sources concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke.

The risk of thromboembolic and thrombotic diseases associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders. Should any occur or be suspected, the drug should be discontinued immediately.

A fourfold to sixfold increased risk of postsurgery thromboembolic complications has been reported in users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

2. *Ocular lesions:* Neuro-ocular lesions, such as optic neuritis or retinal thrombosis, have been associated with the use of oral contraceptives. Discontinue the oral contraceptive if there is unexplained sudden or gradual, partial, or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions.

3. *Carcinoma:* Long-term continuous administration of estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver.

In humans, an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women has been reported. However, there is no evidence suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives.

Studies found no evidence of increase in breast cancer in women taking oral contraceptives; however, an excess risk in users with documented benign breast disease was reported.

There is no confirmed evidence of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of users is, nevertheless, essential. In cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer, or who have breast nodules, fibrocystic disease, or abnormal mammograms, should be monitored with particular care.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with oral contraceptives. Because hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage, they should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock.

A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Usage in or Immediately Preceding Pregnancy; Birth Defects in Offspring, and Malignancy in Female Offspring:* During early pregnancy, female sex hormones may seriously damage the offspring.

An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of oral contraceptives in pregnancy.

There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives.

Pregnancy should be ruled out before continuing an oral contraceptive in any patient who has missed two consecutive menstrual periods. If the patient has not adhered to the schedule, the possibility of pregnancy should be considered at the time of the first missed period, and oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed.

Women who discontinue oral contraceptives with the intent of becoming pregnant should use an alternate form of contraception for a period of time before attempting to conceive.

Administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives.

7. *Carbohydrate and Lipid Metabolic Effects:* Because decreased glucose tolerance has been observed in a significant percentage of patients, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives.

An increase in triglycerides and total phospholipids has been observed.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving oral contraceptives. The prevalence in users increases with longer exposure. Age is also strongly correlated with development of hypertension. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure.

9. *Headache:* Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, nonfunctional causes should be borne in mind. In undiagnosed abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy.

Women with a past history of oligomenorrhea or secondary amenorrhea, or young women without regular cycles should be advised that they may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast Feeding:* Oral contraceptives may interfere with lactation. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs.

PRECAUTIONS

1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another examination.

2. Preexisting uterine leiomyomata may increase in size.

3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

4. Oral contraceptives may cause fluid retention and should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice. If jaundice develops, the medication should be discontinued.

6. Steroid hormones may be poorly metabolized and should be administered with caution in patients with impaired liver function.

7. Users may have disturbances in normal tryptophan metabolism, which may result in a relative pyridoxine deficiency.

8. Serum folate levels may be depressed.

9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted.

10. Certain endocrine and liver function tests and blood components may be affected.

(a) Increased sulfobromophthalein retention. (b) Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. (c) Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone. (d) Decreased pregnanediol excretion. (e) Reduced response to metyrapone test.

Drug interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin and an association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and ampicillin.

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with oral contraceptives: thrombophlebitis; pulmonary embolism; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; benign hepatomas; congenital anomalies.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, eg, retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms; breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma; breast changes; change in weight; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately post partum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature; intolerance to contact lenses.

The following adverse reactions have been reported and the association has been neither confirmed nor refuted: premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis; porphyria.

Special Notes on Administration

Norlestrin [Fe] 1/50 (norethindrone acetate and ethinyl estradiol tablets, USP) and Norlestrin [28] 1/50—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after the brown ferrous fumarate or white inert tablets have been started.

Norlestrin [21] 1/50—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after discontinuing medication.

After several months on treatment, bleeding may be reduced to a point of virtual absence; reduced flow may be a result of medication and not indicative of pregnancy.

HOW SUPPLIED

Norlestrin [Fe] 1/50 is available in minicompacts each containing 21 yellow tablets and 7 brown tablets. Each yellow tablet contains 1 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Each brown tablet contains 75 mg of ferrous fumarate. Available in packages of five minicompacts and packages of five refills.

Norlestrin [28] 1/50 is available in minicompacts each containing 21 yellow tablets and 7 white inert tablets. Each yellow tablet contains 1 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Available in packages of five minicompacts and in packages of five refills.

Norlestrin [21] 1/50 is available in minicompacts each containing 21 yellow tablets. Each yellow tablet contains 1 mg of norethindrone acetate and 50 mcg of ethinyl estradiol. Available in packages of five minicompacts and packages of five refills.

YB

PARKE-DAVIS
Div of Warner-Lambert Co
Morris Plains, NJ 07950 USA

PD-JA-0270-1-P (3-80)

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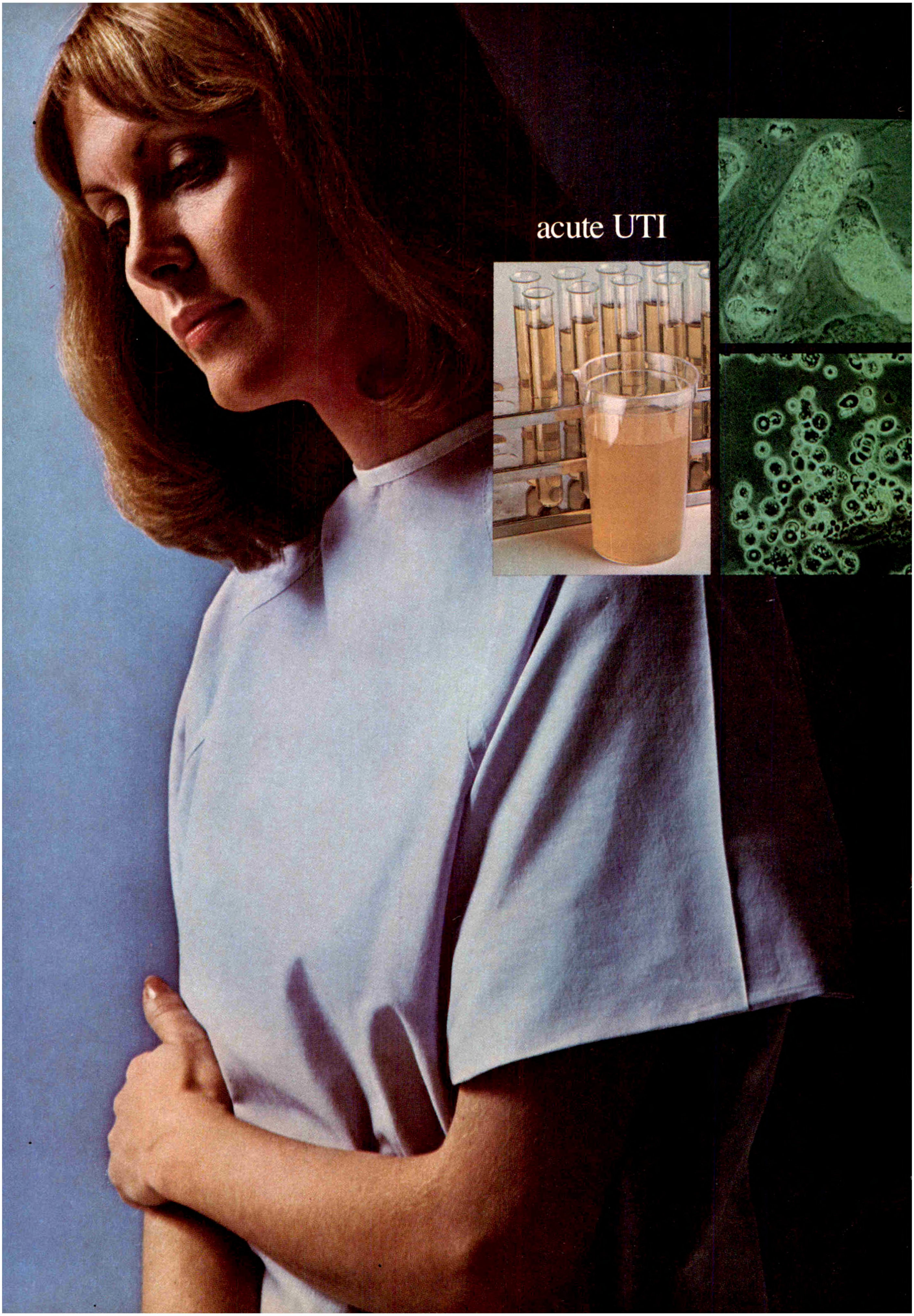
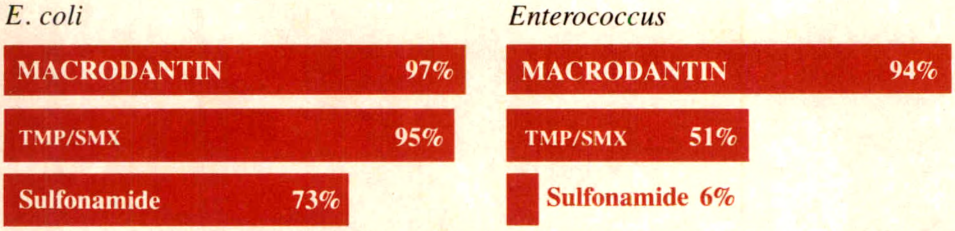

acute UTI

In acute UTI

When your first choice is Macrodantin® you'll rarely need a second one

Macrodantin is potent against both major uropathogens in office practice—***E. coli***, the most common, and ***enterococcus***—unlike other widely prescribed antimicrobials used individually or in combination.

Superior in vitro* against the two most common uropathogens[1]



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*In vitro data do not necessarily predict clinical efficacy.

[1] PMR Bacteriologic Report, Summer Series 1979;
a national bacteriologic monitoring service for 200 acute-care hospitals of 100 beds or more.

Macrodantin remains consistently effective year after year—resistance is negligible

- Does not foster resistant bowel flora which may recycle infection.
- Does not change normal vaginal flora... little risk of Candida overgrowth.
- Concentrates its action in the urinary tract.

Lets you reserve systemic agents for systemic infection.

...too valuable to keep in reserve

Macrodantin® Capsules: 25,50,100mg
(nitrofurantoin macrocrystals)

Norwich-Eaton
PHARMACEUTICALS

Norwich, New York 13815
Division of MortonNorwich Products, Inc.

See next page for a brief summary of prescribing information including susceptible organisms.

. . . too valuable to keep in reserve

Macrodantin®

(nitrofurantoin macrocrystals)

Capsules: 25, 50, 100mg

INDICATIONS: Macrodantin is indicated for the treatment of urinary tract infections when due to susceptible strains of *Escherichia coli*, enterococci, *Staphylococcus aureus* (it is not indicated for the treatment of associated renal cortical or perinephric abscesses), and certain susceptible strains of *Klebsiella* species, *Enterobacter* species, and *Proteus* species.

NOTE: Specimens for culture and susceptibility testing should be obtained prior to and during drug administration.

CONTRAINDICATIONS: Anuria, oliguria, or significant impairment of renal function (creatinine clearance under 40 ml per minute) are contraindications to therapy with this drug. Treatment of this type of patient carries an increased risk of toxicity because of impaired excretion of the drug. For the same reason, this drug is much less effective under these circumstances.

The drug is contraindicated in pregnant patients at term as well as in infants under one month of age because of the possibility of hemolytic anemia due to immature enzyme systems (glutathione instability).

The drug is also contraindicated in those patients with known hypersensitivity to Macrodantin, Furadantin® (nitrofurantoin), and other nitrofurantoin preparations.

WARNINGS: Acute, subacute and chronic pulmonary reactions have been observed in patients treated with nitrofurantoin products. If these reactions occur, the drug should be withdrawn and appropriate measures should be taken.

An insidious onset of pulmonary reactions (diffuse interstitial pneumonitis or pulmonary fibrosis, or both) in patients on long-term therapy warrants close monitoring of these patients.

There have been isolated reports giving pulmonary reactions as a contributing cause of death. (See Hypersensitivity reactions.)

Cases of hemolytic anemia of the primaquine sensitivity type have been induced by Macrodantin. The hemolysis appears to be linked to a glucose-6-phosphate dehydrogenase deficiency in the red blood cells of the affected patients. This deficiency is found in 10 percent of Negroes and a small percentage of ethnic groups of Mediterranean and Near-Eastern origin. Any sign of hemolysis is an indication to discontinue the drug. Hemolysis ceases when the drug is withdrawn.

Pseudomonas is the organism most commonly implicated in superinfections in patients treated with Macrodantin.

Hepatitis, including chronic active hepatitis, has been observed rarely. Fatalities have been reported. The mechanism appears to be of an idiosyncratic hypersensitive type.

PRECAUTIONS: Peripheral neuropathy may occur with Macrodantin therapy; this may become severe or irreversible. Fatalities have been reported. Predisposing conditions such as renal impairment (creatinine clearance under 40 ml per minute), anemia, diabetes, electrolyte imbalance, vitamin B deficiency, and debilitating disease may enhance such occurrence.

Usage in Pregnancy: The safety of Macrodantin during pregnancy and lactation has not been established. Use of this drug in women of childbearing potential requires that the anticipated benefit be weighed against the possible risks.

ADVERSE REACTIONS: Gastrointestinal reactions: Anorexia, nausea and emesis are the most frequent reactions; abdominal pain and diarrhea occur less frequently. These dose-related toxicity reactions can be minimized by reduction of dosage, especially in the female patient. Hepatitis occurs rarely.

Hypersensitivity reactions: Pulmonary sensitivity reactions may occur, which can be acute, subacute, or chronic.

Acute reactions are commonly manifested by fever, chills, cough, chest pain, dyspnea, pulmonary infiltration with consolidation or pleural effusion on x-ray, and eosinophilia. The acute reactions usually occur within the first week of treatment and are reversible with cessation of therapy. Resolution may be dramatic.

In subacute reactions, fever and eosinophilia are observed less often. Recovery is somewhat slower, perhaps as long as several months. If the symptoms are not recognized as being drug related and nitrofurantoin is not withdrawn, symptoms may become more severe.

Chronic pulmonary reactions are more likely to occur in patients who have been on continuous nitrofurantoin therapy for six months or longer. The insidious onset of malaise, dyspnea on exertion, cough, and altered pulmonary function are common manifestations. Roentgenographic and histologic findings of diffuse interstitial pneumonitis or fibrosis, or both, are also common manifestations. Fever is rarely prominent.

The severity of these chronic pulmonary reactions and the degree of their resolution appear to be related to the duration of therapy after the first clinical signs appear. Pulmonary function may be permanently impaired even after cessation of nitrofurantoin therapy. This risk is greater when pulmonary reactions are not recognized early.

Dermatologic reactions: Maculopapular, erythematous, or eczematous eruption, pruritus, urticaria, and angioedema.

Other hypersensitivity reactions: Anaphylaxis, asthmatic attack in patients with history of asthma, cholestatic jaundice, hepatitis, including chronic active hepatitis, drug fever, and arthralgia.

Hematologic reactions: Hemolytic anemia, granulocytopenia, leukopenia, eosinophilia, and megaloblastic anemia. Return of the blood picture to normal has followed cessation of therapy.

Neurological reactions: Peripheral neuropathy, headache, dizziness, nystagmus, and drowsiness.

Miscellaneous reactions: Transient alopecia. As with other antimicrobial agents, superinfections by resistant organisms may occur. With Macrodantin, however, these are limited to the genitourinary tract because suppression of normal bacterial flora elsewhere in the body does not occur.

Eaton Laboratories Inc.
A subsidiary of MortonNorwich Products, Inc., Manatí, Puerto Rico 00701
Address medical inquiries to: Norwich-Eaton Pharmaceuticals, Medical Department, Norwich, New York 13815

Motrin® (ibuprofen)

effective relief of mild to moderate menstrual pain...

Motrin® Tablets (ibuprofen, Upjohn)

Indications and Usage: Relief of mild to moderate pain.

Treatment of signs and symptoms of rheumatoid arthritis and osteoarthritis during acute flares and in long-term management. Safety and efficacy have not been established in Functional Class IV rheumatoid arthritis.

Contraindications: Individuals hypersensitive to it, or with the syndrome of nasal polyps, angioedema, and bronchospastic reactivity to aspirin or other nonsteroidal anti-inflammatory agents (see WARNINGS).

Warnings: Anaphylactoid reactions have occurred in patients with aspirin hypersensitivity (see CONTRAINDICATIONS).

Peptic ulceration and gastrointestinal bleeding, sometimes severe, have been reported. Ulceration, perforation, and bleeding may end fatally. An association has not been established. Motrin should be given under close supervision to patients with a history of upper gastrointestinal tract disease, only after consulting ADVERSE REACTIONS.

In patients with active peptic ulcer and active rheumatoid arthritis, nonulcerogenic drugs, such as gold, should be tried. If Motrin must be given, the patient should be under close supervision for signs of ulcer perforation or gastrointestinal bleeding.

Precautions: Blurred and/or diminished vision, scotomata, and/or changes in color vision have been reported. If these develop, discontinue Motrin and the patient should have an ophthalmologic examination, including central visual fields.

Fluid retention and edema have been associated with Motrin; use with caution in patients with a history of cardiac decompensation.

Motrin can inhibit platelet aggregation and prolong bleeding time. Use with caution in persons with intrinsic coagulation defects and those on anticoagulant therapy.

Patients should report signs or symptoms of gastrointestinal ulceration or bleeding, blurred vision or other eye symptoms, skin rash, weight gain, or edema.

To avoid exacerbation of disease or adrenal insufficiency, patients on prolonged corticosteroid therapy should have therapy tapered slowly when Motrin is added.

Drug interactions. *Aspirin:* Used concomitantly may decrease Motrin blood levels. *Coumarin:* Bleeding has been reported in patients taking Motrin and coumarin.

Pregnancy and nursing mothers: Motrin should not be taken during pregnancy nor by nursing mothers.

Adverse Reactions

Incidence greater than 1%

Gastrointestinal: The most frequent type of adverse reaction occurring with Motrin is gastrointestinal (4% to 16%). This includes nausea,* epigastric pain,* heartburn,* diarrhea, abdominal distress, nausea and vomiting, indigestion, constipation, abdominal cramps or pain, fullness of the GI tract (bloating and flatulence). **Central Nervous System:** Dizziness,* headache, nervousness. **Dermatologic:** Rash* (including maculopapular type), pruritus. **Special Senses:** Tinnitus. **Metabolic:** Decreased appetite, edema, fluid retention. Fluid retention generally responds promptly to drug discontinuation (see PRECAUTIONS).

*Incidence 3% to 9%.

Incidence less than 1 in 100

Gastrointestinal: Upper GI ulcer with bleeding and/or perforation, hemorrhage, melena. **Central Nervous System:** Depression, insomnia. **Dermatologic:** Vesiculobullous eruptions, urticaria, erythema multiforme. **Cardiovascular:** Congestive heart failure in patients with marginal cardiac function, elevated blood pressure. **Special Senses:** Amblyopia (see PRECAUTIONS). **Hematologic:** Leukopenia, decreased hemoglobin and hematocrit.

Causal relationship unknown

Gastrointestinal: Hepatitis, jaundice, abnormal liver function. **Central Nervous System:** Paresthesias, hallucinations, dream abnormalities. **Dermatologic:** Alopecia, Stevens-Johnson syndrome. **Special Senses:** Conjunctivitis, diplopia, optic neuritis. **Hematologic:** Hemolytic anemia, thrombocytopenia, granulocytopenia, bleeding episodes. **Allergic:** Fever, serum sickness, lupus erythematosus syndrome. **Endocrine:** Gynecomastia, hypoglycemia. **Cardiovascular:** Arrhythmias. **Renal:** Decreased creatinine clearance, polyuria, azotemia.

Overdosage: In cases of acute overdosage, the stomach should be emptied. The drug is acidic and excreted in the urine, so alkaline diuresis may be beneficial.

Dosage and Administration: Mild to moderate pain: 400 mg every 4 to 6 hours as necessary for relief of pain.

Rheumatoid arthritis and osteoarthritis, including flares of chronic disease: Suggested dosage is 300, 400, or 600 mg t.i.d. or q.i.d.

Do not exceed 2400 mg per day.

Caution: Federal law prohibits dispensing without prescription.

For additional product information, see your Upjohn representative or consult the package insert.

Upjohn THE UPJOHN COMPANY
Kalamazoo, Michigan 49001 USA

MED B-4-S

J-7783-4

September 1980

effective, well-tolerated, nonhormonal relief of mild to moderate menstrual pain

Motrin® 400 mg TABLETS

ibuprofen, Upjohn

reduces uterine hypertonicity... relieves pain rapidly... by inhibiting prostaglandin synthesis

- more effective than aspirin for relief of menstrual pain
- better tolerated than aspirin in the GI tract
- nonnarcotic
- most common side effect, mild gastrointestinal disturbance
- one 400 mg tablet q4-6h when pain and cramps occur

For a brief summary of prescribing information see facing page.

Upjohn THE UPJOHN COMPANY
Kalamazoo, Michigan 49001 USA

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approaching
12 million woman-years
of experience

21- and 28-day regimens

Ortho-Novum 1/50
TRADEMARK

Each yellow tablet* contains 1 mg norethindrone and 0.05 mg mestranol.
Each green tablet in the 28-day regimen contains inert ingredients.
*Contains FD&C Yellow No. 5 (tartrazine) as a color additive.

a trusted leader
in low-dose oral contraception

Please see complete prescribing information,
a brief summary of which appears on the next page.

ORTHO

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

Serious as well as minor side effects have been reported with the use of oral contraceptives. These include thromboembolic disease. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. The physician should be fully aware of the Complete Prescribing Information for this product.

Pharmaceutical Corporation 1980

[illegible] complete prescribing information and patient information before prescribing the product.

INDICATION: CONTRACEPTION. The pregnancy rate in women using conventional combination oral contraceptives (containing 35 mcg or more of ethinyl estradiol or 50 mcg or more of mestranol) is generally reported as less than one pregnancy per 100 woman-years of use. Slightly higher rates (somewhat more than one pregnancy per 100 woman-years of use) are reported for some combination products containing 35 mcg or less of ethinyl estradiol, and rates on the order of three pregnancies per 100 woman-years are reported for the progestogen-only oral contraceptives. Table 1 gives ranges of pregnancy rates reported in the literature for other means of contraception. The efficacy of these means of contraception (except the IUD) depends upon the degree of adherence to the method.

Table 1: Pregnancies Per 100 Women-Years. IUD, less than 1-6, Diaphragm with spermicidal product (creams or jellies), 2-20, Condom, 3-36, Aerosol foams, 2-29, Jellies and creams, 4-36, Periodic abstinence (rhythm) all types, less than 1-47, 1. Calendar method, 14-47; 2. Temperature method, 1-20; 3. Temperature method—intercourse only in postovulatory phase, less than 1-7; 4. Mucus method, 1-25; No contraception, 60-80. **DOSE-RELATED RISK OF THROMBOEMBOLISM FROM ORAL CONTRACEPTIVES:** Two studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of oral contraceptives be started on preparations containing .05 mg or less of estrogen. **CONTRAINDICATIONS:** Oral contraceptives should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed, abnormal genital bleeding. 7. Known or suspected pregnancy (see WARNINGS, No. 5). 8. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen-containing products.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.
The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Four principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. Overall excess mortality due to pulmonary embolism or stroke is on the order of 1.0 to 3.5 deaths annually per 100,000 users and increases with age. **CEREBROVASCULAR DISORDERS:** In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than in nonusers and the risk of thrombotic stroke was 4.0 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION: An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia), the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. The annual excess case rate (increased risk) of myocardial infarction (fatal and nonfatal) in oral contraceptive users was estimated to be approximately 7 cases per 100,000 women users in the 30-39 age group and 67 cases per 100,000 women users in the 40-44 age group. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, but about a 10-to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent. **Risk of Dose:** In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only oral contraceptives have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. The risk of thromboembolic and thrombotic disorders, in both users and nonusers of oral contraceptives, increases with age. Oral contraceptives are, however, an independent risk factor for these events. **ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES:** A large prospective study carried out in the United Kingdom estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of oral contraceptives according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous versus intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for five or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for five or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. Another study published at the same time confirms a previously reported increase of mortality in pill users from cardiovascular disease. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of oral contraceptives) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The findings of this analysis are shown in Figure 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers [more than 15 cigarettes a day], the rates given would be about double; for "light" smokers [less than 15 cigarettes a day], about 50 percent.) The mortality associated with oral contraceptive use in nonsmokers over 40 is higher than with any other method of contraception in that age group. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia and especially by cigarette smoking. The risk of myocardial infarction in oral contraceptive users is substantially increased in women age 40 and over, especially those with other risk factors. Based on the data currently available, the following chart gives a gross estimate of the risk of death from circulatory disorders associated with the use of oral contraceptives.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS—RISK ASSOCIATED WITH USE OF ORAL CONTRACEPTIVES

Age	Below 30	30-39	40+	
Heavy smokers	C	B	A	A—Use associated with very high risk
Light smokers	D	C	B	B—Use associated with high risk
Nonsmokers (no predisposing conditions)	D	C,D	C	C—Use associated with moderate risk
Nonsmokers (other predisposing conditions)	C	C,B	B,A	D—Use associated with low risk

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of postsurgery thromboembolic complications has been reported in oral contraceptive users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2. OCULAR LESIONS. There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue oral contraceptive medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions and institute appropriate diagnostic and therapeutic measures. 3. CARCINOMA. Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in oral contraceptives, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on oral contraceptives. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time oral contraceptives were first given, polycystic ovaries), nearly all occurred in women who had used a sequential oral contraceptive. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives. Several studies have found no increases in breast cancer in women taking oral contraceptives or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with oral contraceptives, found an excess risk in the subgroups of oral contraceptive users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of oral contraceptives has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception.

*Trademark

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	19.3	21.9
Abortion only	2.3	2.5	2.5	5.2	9.8	6.6
Pill only-nonsmokers	1.3	1.4	1.4	2.2	4.5	3.1
Pill only-smokers	1.5	1.6	1.6	10.8	13.4	58.9
IUDs only	1.1	1.2	1.2	1.4	1.6	1.4
Traditional contraception only	1.1	1.4	1.9	3.7	4.7	4.0
Traditional contraception and abortion	0.3	0.4	0.4	0.8	1.4	0.8

4. HEPATIC TUMORS. Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study showed that oral contraceptive formulations with high hormonal potency were associated with a higher risk than lower potency formulations and use of oral contraceptives with high hormonal potency and age over 30 years may further increase the woman's risk of hepatocellular adenoma. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of oral contraceptives. Two studies relate risk with duration of use of the contraceptive, the risk being much greater after four or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5. USE IN OR IMMEDIATELY PRECEDING PREGNANCY, BIRTH DEFECTS IN OFFSPRING, AND MALIGNANCY IN FEMALE OFFSPRING. The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be on the order of 1 to 4 in 1000 exposures. Although there is no evidence at the present time that oral contraceptives further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use oral contraceptives instead of other methods of contraception. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including oral contraceptives, in pregnancy. One case control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if the progestogen-only oral contraceptives are used), and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. GALLBLADDER DISEASE. Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives and estrogens. In one study, an increased risk appeared after two years of use and doubled after four or five years of use. In one of the other studies, an increased risk was apparent between six and twelve months of use. 7. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. An increase in triglycerides and total phospholipids has been observed in patients receiving oral contraceptives. The clinical significance of this finding remains to be defined. 8. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in patients receiving oral contraceptives. In some women hypertension may occur within a few months of beginning oral contraceptive use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in oral contraceptive users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. Hypertension that develops as a result of taking oral contraceptives usually returns to normal after discontinuing the drug. 9. HEADACHE. The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives and evaluation of the cause. 10. BLEEDING IRREGULARITIES. Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Postuse anovulation, possibly prolonged, may also occur in women without previous irregularities. 11. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. 12. BREAST FEEDING. Oral contraceptives given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of oral contraceptives should be deferred until the infant has been weaned. **PRECAUTIONS: General:** 1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by oral contraceptive therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping oral contraceptives, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. **INFORMATION FOR THE PATIENT: (See Patient Package Insert). DRUG INTERACTIONS:** Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin, and tetracycline. **CARCINOGENESIS, PREGNANCY, NURSING MOTHERS: See CONTRAINDICATIONS and WARNINGS. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS): Thrombophlebitis. Pulmonary embolism. Coronary thrombosis. Cerebral thrombosis. Cerebral hemorrhage. Hypertension. Gallbladder disease. Liver tumors. Congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis. Neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related. Nausea, usually the most common adverse reaction. Vomiting, occurs in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Dysmenorrhea. Amenorrhea during and after treatment. Temporary infertility after discontinuance of treatment. Edema. Chloasma or melasma which may persist. Breast changes: tenderness, enlargement, and secretion. Change in weight (increase or decrease). Change in cervical erosion and cervical secretion. Possible diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Increase in size of uterine leiomyomata. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome. Cataracts. Changes in libido. Chorea. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. (ORTHO-NOVUM 1/50□21 and ORTHO-NOVUM 1/50□28 contain tartrazine. Allergic reactions have been reported with the ingestion of this dye in some patients.) **ACUTE OVERDOSE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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Revised 6/80

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American Journal of Obstetrics and Gynecology

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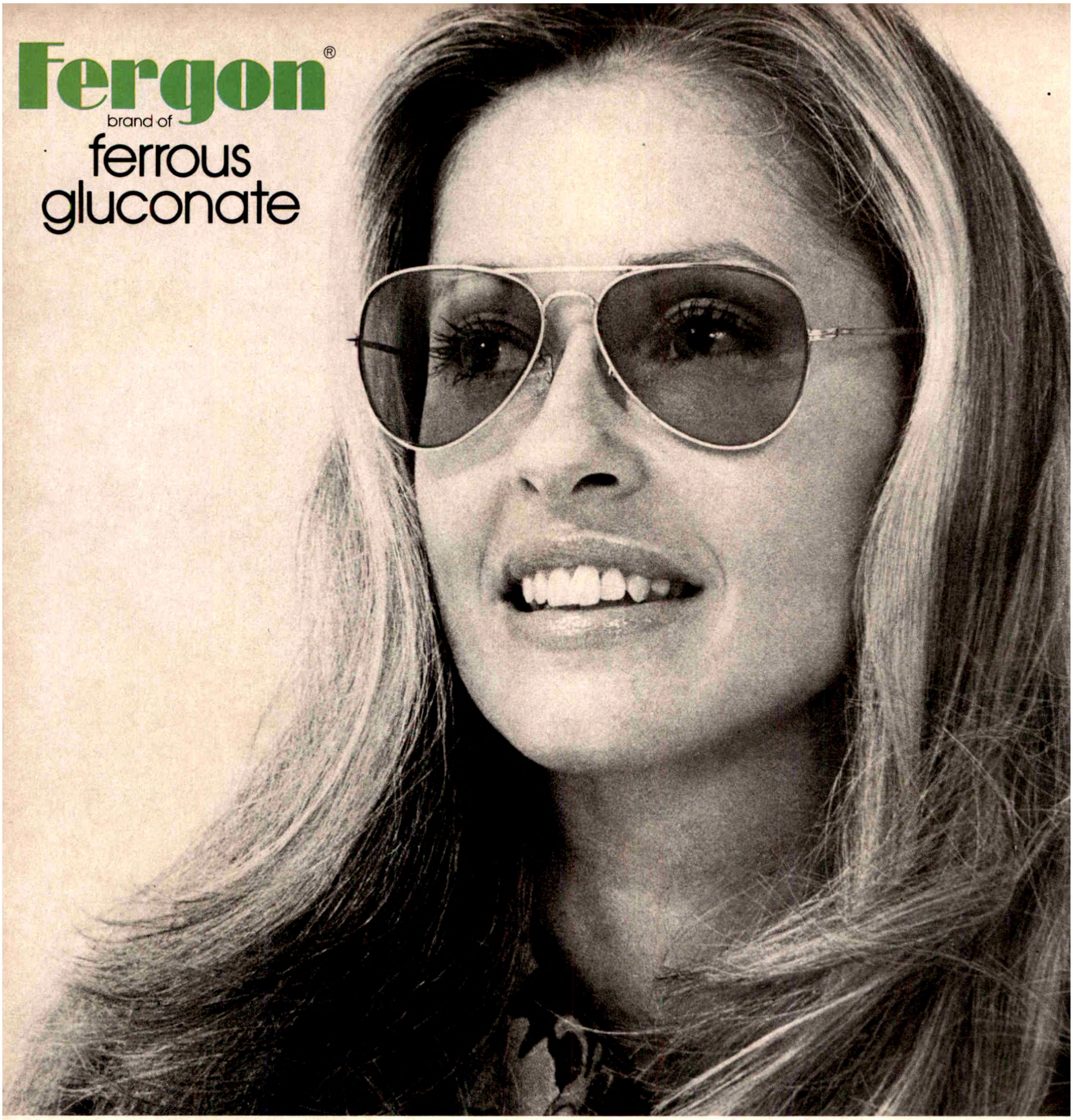

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American Journal of Obstetrics and Gynecology

volume 138 *number* 6 NOVEMBER 15, 1980

Papers of the Society for Gynecologic Investigation

Peroxidase activity in glands and stroma of human endometrium

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Peroxidase activity was measured in specimens of human endometrium taken at different days of the menstrual cycle. High specific activities were more frequently found in secretory endometrium than in proliferative tissue, but the variance at each phase of the cycle was large and differences were statistically not significant. The results obtained do not support the hypothesis that peroxidase activity is determined by the levels of circulating estrogens, as is apparently true in rats. Extremely high levels of peroxidase activity were noticed in some, but not all, specimens of endometrial adenocarcinoma. Endometrial glands were separated from the stroma after collagenase digestion of normal and abnormal endometrium, and peroxidase activity was measured in each fraction. The enzyme was found to be preferentially localized in the stroma, in contrast to the reported findings of predominantly epithelial localization in the rat. (AM. J. OBSTET. GYNECOL. 138:599, 1980.)

LUCAS and co-workers[1] reported, in 1955, that the specific activity of uterine peroxidase in the rat could be elevated up to 200-fold by treatment of the ovariectomized animals with estradiol or diethylstilbestrol. Other studies have confirmed these findings[2-6] and revealed that estrogens provoke extraordinary increases in uterine peroxidase activity also in mice.[7] Responses in guinea pig and hamster uteri were considerably smaller. Stimulation of rat uterine peroxidase by estrogens could be prevented by simultaneous administration of antiestrogens and progesterone.[8] Histochemical studies have indicated that the activity is localized in the luminal and glandular epithelium of the rat uterus.[2, 4, 9]

A wide range of peroxidase activity was found in human endometrium by Lucas and associates[10] and by Lyttle and De Sombre.[7] According to one of these reports,[10] the activity increases during the luteal phase and reaches a maximum at days 17 to 20 of the menstrual cycle. This finding indicates that human endometrial peroxidase and the murine uterine enzyme may differ in their response to ovarian hormones.

The purpose of the present study was to reexamine the levels of peroxidase in human endometrium dur-

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Supported by Grant No. CA 15648 from the National Cancer Institute, and Grant No. HD 07197 from the National Institutes of Health.

Sponsored by the Society for Gynecologic Investigation.

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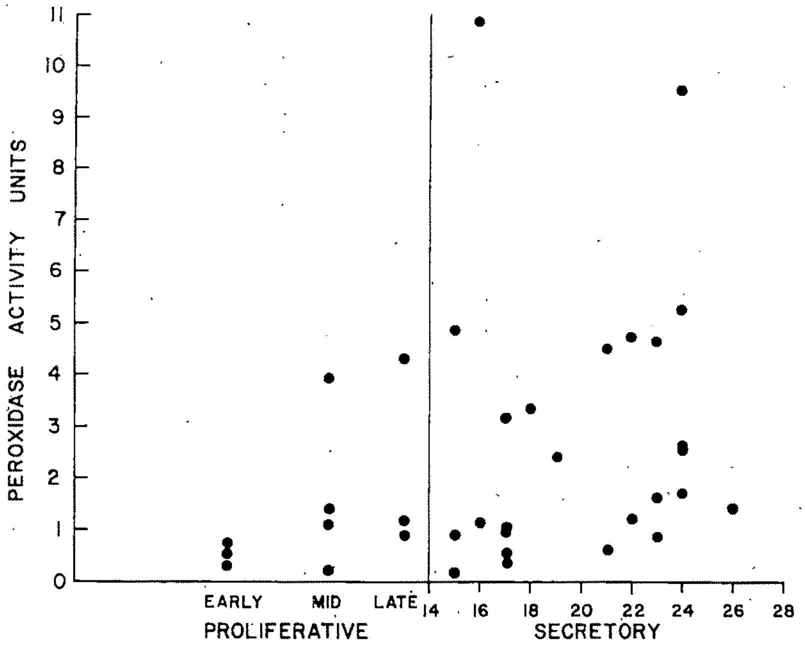

Fig. 1. Peroxidase activity in normal endometrium. Specimens were dated according to the criteria of Noyes and associates.[13] Each unit of enzymatic activity corresponds to the increment of 1 optical density unit per minute per milligram of DNA in the sample from which the enzyme was extracted.

ing the menstrual cycle, by applying the same assay method used to obtain most of the data on activities in the rat uterus. This point of methodology may be of importance in the comparison of hormonal effects on uterine peroxidase activity in different species, since various types of tissue peroxidases have been reported.[11] Activities in specimens of endometrial adenocarcinoma were also measured and compared with the activity in normal endometrium.

Another purpose of this work was to determine the distribution of peroxidase activity in stroma and glandular epithelium isolated from specimens of human endometrium by methods previously reported.[12] Attempts were made to measure peroxidase activity in fragments of endometrium kept in tissue culture, and in monolayer cultures of stromal and epithelial endometrial cells.

The results obtained indicate that conclusions about hormonal dependence and cellular distribution of uterine peroxidase derived from work on rodents cannot be extrapolated to the human endometrium.

Material and methods

Samples of endometrium were obtained by curettage performed for reasons unrelated to this study, or by scraping the uterus after hysterectomy. The specimens were transported to the laboratory in cold Earl's balanced salt solution containing 1% of an antibiotic-antimycotic mixture (GIBCO, Grand Island, New York), and they were used within 4 hours. Dating of the endometrial specimens according to the criteria of Noyes and associates[13] and pathologic evaluation of them were kindly performed by Dr. Liane Deligdisch, Department of Pathology, Mount Sinai School of Medicine.

Glands were separated from stroma in a slight modification of a method previously described in detail.[12] Small pieces of endometrium, approximately 1 cubic millimeter, were digested in Ham's F-10 medium with L-glutamine (Flow Laboratories) containing 0.25% collagenase, type I (Worthington Biochemical Corporation). Digestion was carried out for 1 to 2 hours at 37° C with shaking in a water bath. The digested tissue suspension was subsequently passed through a 38 μm stainless steel sieve (Newark Wire Cloth Co., Newark, New Jersey) to separate glands, retained on the filter, from stromal cells. The two tissue components were collected in Ham's F-10 medium and centrifuged; the pellets were washed in 10 mM Tris-HCl buffer before homogenization.

Peroxidase assay. The enzyme assays were performed essentially according to the procedures described by Lyttle and De Sombre.[6] Approximately 20 to 30 mg of tissue was minced on ice, washed with 10 mM Tris-HCl buffer, pH 7.2, gently homogenized on ice in a glass/glass homogenizer with 0.5 ml of the same buffer, and centrifuged at 105,000 × g for 30 minutes at 2° C. The supernatant, which had no peroxidase activity, was discarded and the pellet was extracted with 0.2 ml of 10 mM Tris-HCl containing 0.5M $CaCl_2$. The homogenate was then transferred to a centrifuge tube with the aid of 0.2 ml of the same buffer and centrifuged at 105,000 × g for 30 minutes at 2° C. The pellet was stored at −30° C for determination of DNA,[14] and the supernatant was used for the assay of peroxidase activity.

Enzyme activity was determined spectrophotometrically, with the use of guaiacol and hydrogen peroxide as substrates in triplicate at 22° C. Fifty microliters of the extract was added to 0.8 ml of freshly prepared substrate solution (10 mM Tris-HCl, pH 7.2, 0.5M $CaCl_2$, 13 mM guaiacol, 0.3 mM H_2O_2) in a 1.5 ml-cuvette, and the initial rate of guaiacol oxidation was determined from the increase in absorbance at 470 nm in a Bausch and Lomb Spectronic 70. A peroxidase activity unit is defined as an activity in the tissue producing an increase of 1 optical density unit per minute per milligram of DNA under the experimental conditions described above.

The same assay procedures were applied to the measurement of peroxidase activity in homogenates of glands or stromal cells, obtained as described above.

Results

Several tests were conducted in order to validate the measurements of peroxidase activity. Linearity of absorbance increments with time and proportionality of the measured activity with enzyme concentration in the assay medium were demonstrated. Only a slight decline in the enzymatic activity was noted in a sample stored on ice for 6 hours.

For the same purpose, the peroxidase activity in erythrocyte ghosts was estimated. Because of the possibility that blood cells might contaminate the samples in spite of repeated washing of the finely minced tissue, the $CaCl_2$ extract of the pellet that remained after hypotonic lysis of cells separated from 10 ml of heparinized blood was subjected to the peroxidase assay. From the values obtained, it was estimated that a 30% contamination of the sample with blood could account for no more than 0.03 unit of peroxidase activity. No significant numbers of eosinophils or other granulocytes containing peroxidase activity were detected by histologic examination of both normal and cancerous specimens stained with hematoxylin and eosin.

Fig. 1 shows the levels of peroxidase activity in endometrium obtained at different days of the menstrual cycle. Low values of peroxidase (0.2 to 2.0 units) were measured both in proliferative and secretory endometrium, but higher activities (2.5 to 11 units) were more frequently found during the luteal phase (12 cases in secretory endometrium versus two in proliferative tissue). Coefficient of correlation analysis between peroxidase activity and day of the menstrual cycle revealed a slight trend toward an increase from the early proliferative to the late secretory phases. The coefficient of correlation was r = 0.30, P = 0.08. No statistical differences between the mean values corresponding to samples of proliferative and secretory endometrium were detected by Student's t test. The wide range of values detected in each phase of the menstrual cycle and the apparent predominance of higher activities in secretory endometrium do not support the hypothesis that estrogens by themselves stimulate peroxidase activity in human endometrium.

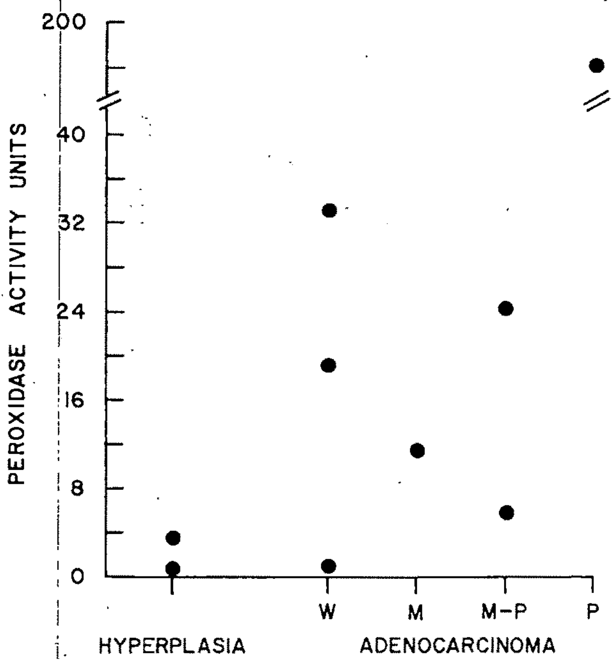

Fig. 2. Peroxidase activity in specimens of hyperplastic endometrium and well-differentiated *(W)*, moderately *(M)*, moderately to poorly *(M-P)*, and poorly *(P)* differentiated endometrial adenocarcinoma.

Fig. 2, drawn on a different scale than Fig. 1, shows levels of peroxidase activity found in a few samples of hyperplastic endometrium and endometrial adenocarcinoma of various degrees of differentiation. The remarkably high values of peroxidase present in four of the seven specimens of endometrial cancer studied were never seen in normal tissue. However, activities in the other three samples of endometrial carcinoma were within the normal range. The small number of samples of endometrial adenocarcinoma that were analyzed does not allow the study of correlations of activities with the degree of differentiation or other characteristics of the tumor. The two cases of endometrial hy-

Table I. Glandular and stromal peroxidase activity in several types of endometrium

Types of endometrium	*Peroxidase activity units*			
	Whole tissue	*Glands*	*Stroma*	*Stroma/glands*
Midproliferative	1.1	0.31	1.2	4
Midproliferative	3.9	0.20	6.4	32
Late proliferative	0.92	~0	2.0	—
Secretory, Day 17	1.0	0.04	1.3	32
Day 21	0.63	0.17	3.3	19
Day 23	4.7	QNS	18	—
Day 24	5.3	0.04	3.8	95
Adenomatous hyperplasia	3.5	0.61	5.1	8
Well-differentiated adenocarcinoma	33	4.3	26	6

QNS = Quantity not sufficient.

perplasia showed activity values within the range of normal proliferative endometrium.

Table I presents values of peroxidase activity in whole endometrium and in the separated stromal and epithelial components. Preferential localization of the activity in the stromal cells is clearly apparent. In most cases, the values of peroxidase activity in whole tissue lie, as would be expected, between the specific activities in glands and stroma.

Since the relative proportion of glandular and stromal cells is, on the average, about 1.3, as determined by measurements of DNA content in glands retained on the filter and in the stromal cells in the filtrate, one can conclude from the data in Table I that the peroxidase activity resides preferentially in the stromal component of the endometrium.

These results contrast with the histochemical findings in estrogen-stimulated rats[4] which indicate that rat uterine peroxidase activity resides in the luminal and glandular epithelium.

Similar preferential localization of peroxidases in stromal cells was found in a specimen of hyperplastic endometrium and in another of well-differentiated endometrial adenocarcinoma, also shown in Table I.

Comment

The peroxidase activity in human endometrium appears to differ from that in the rat uterus, both in hormonal dependence and cellular distribution. The changes in endometrial peroxidase during the menstrual cycle failed to show evidence of stimulation by estrogens. Although some high levels were noted in midproliferative and late-proliferative tissue and at days 15 and 16, when the tissue was at a point of transition between proliferative and secretory phases, others remained low at the end of the follicular phase, and the differences of the means in the two phases were statistically not significant. In human beings, peroxidase activity is preferentially found in the stroma, whereas the enzyme is localized mainly in luminal and glandular epithelial cells in the rat.[2, 4, 9]

Species differences in hormonal control of uterine enzymatic activities are not completely unexpected. Estradiol 17β dehydrogenase, for instance, is stimulated by progestins in human endometrium[15] and by estrogens in rat uterus.[16, 17] More direct experiments, either in vivo or in vitro, are necessary to answer the question of hormonal dependence of endometrial peroxidase activity in human beings. Attempts to study in vitro the effects of various hormones on peroxidase activity in human endometrium were handicapped by the loss of activity in tissue kept in organ culture or in endometrial cells grown in monolayer.

A factor that may be responsible for the marked variance in the peroxidase levels is the age distribution of the patients (51% of the patients were 40 years old or over and only 6% were under 30 years of age). For instance, age-related impairments in uterine response to a deciduogenic stimulus has been demonstrated in mice before midlife.[18]

Peroxidase appears to share with alkaline phosphatase the same pattern of activity throughout the menstrual cycle.[19] As is also the case with alkaline phosphatase, the specificity and function of peroxidase in endometrium are not known.

No distinct pattern emerged of peroxidase activity in cancer tissues. It is of interest that levels of peroxidase in breast cancer also showed great variability,[7] but the spread of that variability was relatively uniform and quantitatively comparable to the distribution of peroxidase activity in normal human endometrium reported in the same study. In contrast, the high levels of peroxidase activity in cancer tissues observed in the present study were markedly above the highest values in normal endometrium.

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Comparison of human and sheep chorion laeve permeability to glucose, β-hydroxybutyrate, and glycerol

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Significant permeability of in vitro human chorion laeve to glucose, glycerol, and β-hydroxybutyrate (βOH butyrate) and in vitro sheep chorion laeve to glycerol and βOH butyrate was measured. Diffusion across these tissues of glycerol and βOH butyrate corresponded to the relative molecular size, charge, and lipid solubility of these compounds. The simple diffusion of glucose across human chorion laeve was somewhat reduced by metabolic conversion of this compound during the transfer process. Demonstration of in vitro human placental tissue permeability to ketoacids and glycerol suggests that these compounds cross to the fetus when elevated in the maternal diabetic state. (AM. J. OBSTET. GYNECOL. 138:604, 1980.)

INCREASES in the maternal levels of ketoacids and glycerol frequently accompany hyperglycemia in the poorly controlled pregnant diabetic patient.[1] It is often assumed that fetal ketoacidosis occurs secondary to the maternal accumulation of ketoacids under these conditions. However, evidence for this assumption in human beings is essentially limited to the demonstration of increased fetal ketoacids during periods of maternal fasting in nondiabetic patients.[2-6] Both fetal and maternal compartments are hypoglycemic and hypoinsulinemic during maternal fasting, and, thus, fetal elevations of ketoacids could have originated from fetal lipolysis. Data from other species are conflicting, in that ketoacids appear to cross the placenta in rodents but do not appear to penetrate placental tissue in ungulates.[7] There is very little experimental data on placental permeability to glycerol in any species. In an attempt to provide further information in regard to placental permeability to ketoacids, the present study was undertaken to measure the permeability of human and sheep placental tissue, mounted in vitro, to β-hydroxybutyrate (βOH butyrate), glycerol, and glucose.

Methods

Chorion laeve obtained from 68 human and 11 sheep pregnancies were studied in separate experiments. All membranes were mounted in an in vitro diffusion chamber within 10 minutes of delivery, as previously described.[8, 9] The mounted membranes divided the chamber into two compartments, both of which communicated with a chimney posteriorly. A gas mixture that contained 95% oxygen and 5% carbon dioxide was bubbled into the base of the chimneys in order to provide oxygen requirements and maintain the pH of a 20 millimolar bicarbonate solution between 7.4 and 7.5. The bathing medium contained physiologic quantities of other electrolytes and glucose. These experiments were performed at 38° C by suspension of the diffusion chambers in a constant-temperature water bath.

Concentration differences across the mounted tissue layers were created by the addition of 200 mg/dl of

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Supported in part by United States Public Health Service Grant IPOL-HD-11725-01.

Presented at the Twenty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 21-24, 1979.

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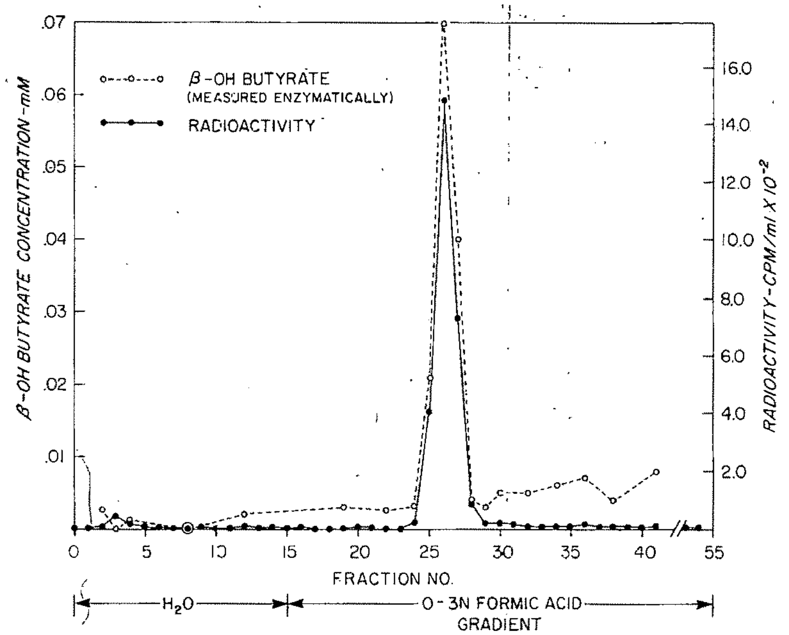

Fig. 1. Elution profiles for nonradioactive and radioactive β-hydroxybutyrate on 1 by 24 cm anion exchange (formate form) Dowex 1 – ×8 column. After introduction of these samples, the column was eluted with a 3N formic acid gradient. Ninety-seven percent of the radioactivity (C^{14}–βOH butyrate) emerged in fractions 25-29, which also contained the major amount of cold βOH butyrate, thus indicating little chemical alteration of the radiolabeled βOH butyrate during diffusion across this tissue.

glucose, 0.1 μCi of C^{14}-labeled β-hydroxybutyrate, or 0.1 μCi of C^{14}-labeled glycerol into the chimney on one side of the system. Each test solute was studied individually, in separate experiments. In certain cases, a 10 mg/dl paraminohippurate gradient was created simultaneously to provide a reference solute of similar size and water solubility for comparison of placental permeability. Permeability constants (cm sec^{-1}) and diffusion coefficients (cm^2 sec^{-1}) were calculated for each experiment from serial measurements of changes in solute concentration or radioactivity over 90 minutes on the two sides of the chamber. Diffusion coefficients were estimated by determination of the weight of a known area of membrane and assumption of a specific gravity of 1.0. The calculations necessary for such flux experiments have been described previously.[9]

The chemical integrity of C^{14}-radiolabeled βOH butyrate, glycerol, and glucose during these experiments was confirmed by ion exchange chromatography with a (formate form) Dowex column; labeled and enzymatically determined cold βOH butyrate in strong-side and weak-side bathing media from the diffusion chamber was adjusted to pH 6 with 1N hydrochloric acid and eluted with a 3N formic acid gradient (Fig. 1). Radioactive tracer and the enzymatically measured βOH butyrate emerged from the column in essentially identical fractions, thus indicating no significant chemical alteration in βOH butyrate during the diffusion process.

C^{14}-glycerol samples were adjusted to pH 6 and eluted with a 6N formic acid gradient (Fig. 2). Ninety-seven percent of the radioactivity and almost all of the enzymatically determined glycerol appeared in identical fractions, thus indicating that no chemical alteration of the radiolabeled glycerol took place in these experiments.

Samples of C^{14}–D-glucose from a typical diffusion experiment were eluted on a similar column with a 1M NaCl gradient. Elution profiles are presented in Fig. 3. Data from chromatography of bathing media on the weak side of the diffusion chamber at the conclusion of such an experiment indicated that only two thirds of the radioactivity was found in fractions that contained enzymatic glucose activity, whereas one third of the C^{14} appeared in much later fractions, probably representing a glucose metabolite of lesser molecular weight.

Partition coefficients for separation of solute between chloroform and the buffer solutions used in these diffusion experiments were measured as follows: 2 ml of buffer solution that contained a known amount of solute was mixed with 2 ml of chloroform by constant shaking until equilibrium concentrations of solute in the two solvent phases was achieved. No further changes in solute concentration were observed in any

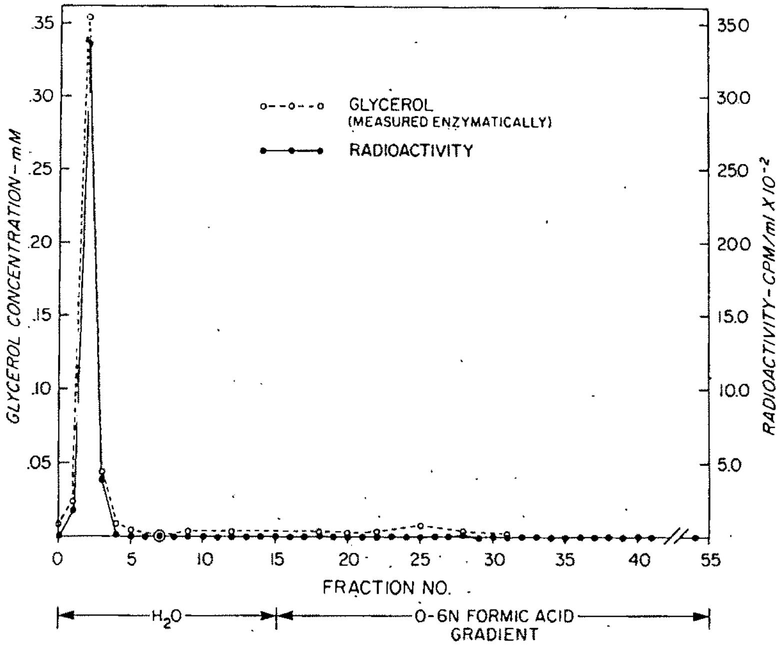

Fig. 2. Elution profiles for C^{14}-radiolabeled glycerol and enzymatically measured nonradiolabeled glycerol on 1 by 26 Dowex 1 − ×8 column. After addition of glycerol samples, the column was eluted with 6N formic acid gradient. Appearance of radioactivity and cold glycerol in identical fractions confirmed the integrity of the radiolabeled glycerol during these experiments.

Table I. Human chorion laeve permeability to glycerol, glucose, and βOH butyrate

Solute	*P × 10⁵ cm sec⁻¹*	*D × 10⁶ cm² sec⁻¹*
Glucose	3.09 ± 0.32 (26)	2.08 ± 0.15 (26)
βOH butyrate	4.10 ± 0.22 (21)	2.97 ± 0.26 (11)
Glycerol	4.91 ± 0.28 (21)	3.13 ± 0.15 (21)

Results given are means ± standard error of the mean. Number of experiments in parentheses.

Table II. Sheep chorion laeve (intercotyledonary) permeability to βOH butyrate and glycerol

Solute	*P × 10⁵ cm sec⁻¹*	*D × 10⁶ cm² sec⁻¹*
βOH butyrate	5.10 ± 0.41 (8)	1.48 ± 0.09 (8)
Glycerol	7.98 ± 0.60 (7)	2.13 ± 0.14 (7)

Results given are means ± standard error of the mean. Number of experiments in parentheses.

sample after 10 minutes. The suspension was centrifuged and solute analysis was performed in triplicate on the separated buffer solution.

Glucose concentration was measured enzymatically. Para-aminohippurate was measured colorimetrically by means of a diazotization reaction described by Bratton and Marshall.[10] C^{14}-βOH butyrate and C^{14}-glycerol activity were counted in a Beckman liquid scintillation counter.

Results

The mean values for human chorion laeve permeability in vitro, at term, are given in Table I. Mean values ± SEM for permeability constants ($P \times 10^{-5}$ cm sec^{-1}) were 3.09 ± 0.32 for glucose, 4.10 ± 0.22 for βOH butyrate, and 4.91 ± 0.28 for glycerol. Mean values ± SEM for diffusion coefficients ($D \times 10^{-6}$ cm^2 sec^{-1}) were 2.08 ± 0.15 for glucose, 2.97 ± 0.26 for βOH butyrate, and 3.13 ± 0.15 for glycerol. When the diffusion of these solutes was measured in vitro across intercotyledonary sheep chorion laeve (Table II), the mean P values ± SEM were 5.10 ± 0.41 for βOH butyrate and 7.98 ± 0.60 for glycerol, and the mean D values were 1.48 ± 0.09 for βOH butyrate and 2.13 ± 0.14 for glycerol. In Table III, in vitro placental tissue permeability to these solutes is compared to simultaneously measured permeability to para-aminohippurate (PAH; molecular weight, 193). PAH is a frequently used standard representing a water-soluble molecule of intermediate molecular size. Ratios of 0.71 for glucose/PAH, 1.08 for βOH butyrate/PAH, and 1.24 for glycerol/PAH were obtained across human placental tissue. With intercotyledonary sheep chorion laeve, values of 1.01 for βOH butyrate/PAH and 1.52 for glycerol/PAH diffusion were obtained. Thus, glycerol diffuses across this tissue more rapidly than PAH, βOH

Table III. Ratio of chorion laeve permeability to various solutes vs. para-aminohippurate

Solute	*Human*	*Sheep*
Glucose	0.71 (26)	
βOH butyrate	1.08 (21)	1.01 (8)
Glycerol	1.24 (21)	1.52 (7)

Number of experiments in parentheses.

butyrate crosses at about the same rate in both species, and glucose crosses at a rate significantly below PAH in the human being.

Data which explain these findings in part are presented in Table IV, where chloroform-water partition coefficients are given for these solutes. All three solutes are essentially water soluble and lipid insoluble, with comparable small chloroform-water partition values corresponding to some similarity in their placental permeability values, that is, they cross this tissue at a rate roughly equivalent to that observed for compounds of similar size that diffuse through extracellular water-filled channels and unaided by solubility in and diffusion through cell lipid. However, the increased size of the glucose molecule and the charge on βOH butyrate (Table IV) probably contribute to somewhat lower diffusion rates. The relatively slower glucose diffusion may also be due to metabolism of this substrate by the membrane. Glucose would appear to diffuse across this tissue at a rate similar to that for PAH, except that, as previously described, approximately one third of this substrate is metabolized during transit.

Membrane permeability to these solutes was unchanged by orientation of the membrane or resulting diffusion direction, that is, maternal-fetal or fetal-maternal (Table V). Permeability values for glucose were constant over 50 to 200 mg/100 ml concentration difference across chorion laeve in separate experiments, indicating that transfer of glucose was linear with gradient. Glucose permeation values were close to those measured for other compounds of similar size, thus indicating no evidence for facilitated diffusion of this molecule across in vitro chorion laeve in these experiments.

Comment

Evidence for significant permeability of human and sheep chorion laeve in vitro to glycerol and βOH butyrate is presented in this study. Both of these solutes were shown to diffuse across this tissue in sizable quantities at rates equal to or in excess of those measured for the similar-sized PAH molecule. These solutes share with PAH a relative low lipid solubility which corresponds to their similar permeation of this membrane. In addition to size and lipid solubility, molecular charge is also known to have an important effect on placental membrane permeability. At pH 7.4, most (99%) of the βOH butyrate molecules (pK 4.4) would be present in dissociated or ionized form, thus retarding the diffusion of this solute across this tissue. For this reason, demonstration of sizable placental permeability to this solute at physiologic pH is of further significance. Glycerol is uncharged, which, along with its smaller molecular size (molecular weight, 92), may account for the relatively large permeation values measured for it.

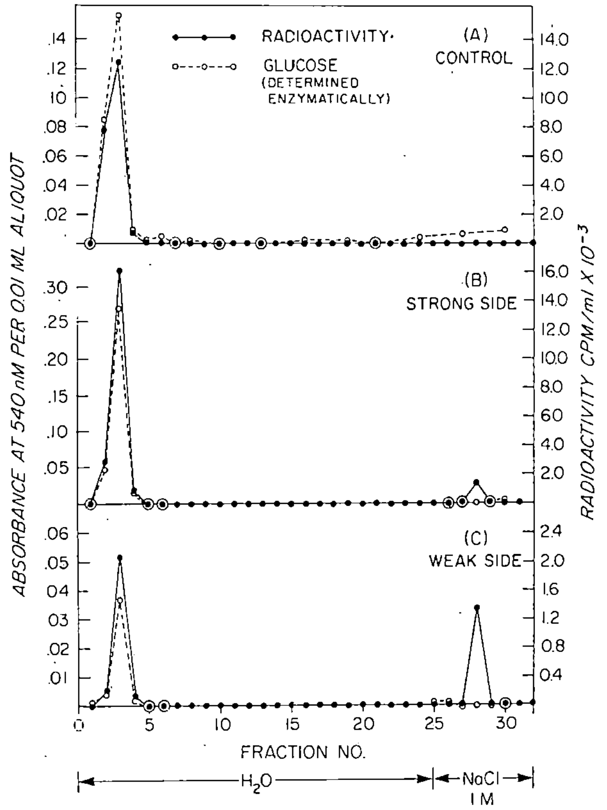

Fig. 3. Elution profiles from a typical diffusion experiment for C^{14}-glucose and nonlabeled glucose on a 1 by 24 cm Dowex 1 – ×8 (formate form) column eluted with a 1M NaCl gradient. Part *A* presents profile for a stock glucose solution showing the radioactive and enzymatically measured compound emerging in identical peaks. Parts *B* and *C* show elution profiles for solutions from strong and weak sides of the diffusion chamber, respectively, at conclusion of experiment. Most significant is the finding that, on the weak side, approximately 35% of the radioactivity emerged separately from the enzymatically determined glucose, indicating that metabolic alteration of approximately one third of the glucose most likely occurred during diffusion of this substrate across this tissue.

Preliminary evidence from fasting sheep preparations by Morriss and co-workers[11] suggested that the

Table IV. Lipid solubility of glycerol, glucose, and βOH butyrate at 37° C

Solute	*Molecular weight*	*Charge at pH 7.4*	*Chloroform/pH 7.4 buffer partition coefficient*
Glucose	180	0	4.2×10^{-4}
βOH butyrate	103	−1	1.4×10^{-2}
Glycerol	92	0	4.3×10^{-4}

Table V. Chorion laeve permeability ($P \times 10^5$ cm sec^{-1}) according to diffusion direction

Solute diffusion direction	*Glucose*	*βOH butyrate*	*Glycerol*
Maternal → fetal	3.2 ± 0.49 (15)	3.67 ± 0.27 (10)	5.15 ± 0.37 (10)
Fetal → maternal	2.92 ± 0.39 (11)	4.49 ± 0.30 (11)	4.70 ± 0.42 (11)

Results given are means ± standard error of the mean. Number of experiments in parentheses. There was no statistically significant difference between permeability values for opposite diffusion directions.

placenta in this species is impermeable in vivo to βOH butyrate. Therefore, the in vitro permeability of chorion laeve to βOH butyrate in this species may not correspond to in vivo events. Disparity between relative placental tissue permeability in vitro and lack of permeability in vivo has been demonstrated by Battaglia and associates[12] for Na^+, Cl^-, and HCO_3^- ions in this species. However, further studies in vivo, with sizable maternal infusions of βOH butyrate, are needed to clarify this issue, since the small transplacental ketoacid gradients during the fasting state are not comparable to the much larger maternal ketoacid accumulation in uncontrolled diabetes.

A strong correlation has been demonstrated between placental tissue permeability measured in vitro and in vivo, to a variety of solutes in the primate.[13-16] Therefore, demonstration of significant diffusion of β-hydroxybutyrate and glycerol across human placental tissue in vitro provides preliminary evidence that these important metabolites, when elevated in the poorly controlled maternal diabetic patient, may cross to the fetus. However, additional in vivo measurements of concentrations of these solutes in cord blood and amniotic fluid during periods of increased maternal levels are necessary to prove conclusively such a transfer.

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Impact of acute surgical stress on anterior pituitary function in female subjects

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The effects of acute surgical stress and the accompanying anesthesia on anterior pituitary function in female subjects was studied by means of frequent perioperative determinations of the circulating concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), growth hormone (GH), and thyroid-stimulating hormone (TSH). No appreciable alteration in the circulating concentrations of either LH or FSH could be detected. In contrast, the levels of PRL and GH were found to be markedly increased during the perioperative period, with the increase in PRL invariably preceding any noticeable alteration in GH levels. In addition, perioperative PRL but not GH levels were significantly higher ($p < 0.01$) in patients undergoing a major surgical procedure, in contrast to a minor one. Finally, this study furnishes detailed evidence of a small but significant ($p < 0.05$) increase in TSH levels in patients undergoing a major surgical procedure. These data indicate that augmentation of pituitary secretion of three stress-related hormones, but not gonadotropins, occurs during surgical procedures. (AM. J. OBSTET. GYNECOL. 138:609, 1980.)

THE INTEGRATED neuroendocrine response to acute stress in man is generally thought to consist of a series of adaptational events designed to restore and maintain overall homeostasis. Such mechanisms can be considered by examining hormonal alterations during surgical procedures. Previous studies of pituitary function during the perioperative period have consistently demonstrated augmentation of growth hormone (GH),[1] prolactin (PRL),[2, 3] and adrenocorticotrophic hormone (ACTH)[4, 5] secretion. However, relatively little information is available with respect to the effect of acute surgical stress on circulating gonadotropin concentrations in female subjects,[6, 7] and studies of perioperative alteration in levels of thyroid-stimulating hormone (TSH) have yielded conflicting results.[6, 8–13] Therefore, the present investigation was undertaken to provide a comprehensive perioperative survey of anterior pituitary function in female subjects by means of frequent simultaneous determinations of five pituitary hormones.

Material and methods

Experimental subjects. Eight women, 22 to 43 years of age, who were scheduled to undergo elective abdominal surgical procedures for benign conditions volunteered for this study, with informed written consent being obtained. All subjects were in good health, apart from the condition that prompted the scheduled operation, and none of the subjects had received any medications for at least 3 months prior to this study. Additional pertinent data are detailed in Table I.

The patients were divided into two groups on the basis of age, underlying diagnosis, and the extent and duration of the surgical procedure. Thus, Patients 1 through 4 were women with normal cycles (mean age, 38 years) who underwent a *major* surgical procedure that lasted approximately 2 hours (123 ± 2 minutes).

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This research was supported by Rockefeller Foundation Grant RF-75029 and National Institutes of Health Research Grant HD-12303.

Sponsored by the Society for Gynecologic Investigation.

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Table I. Characteristics of female subjects undergoing surgical procedures

Patient No.	*Age (yr)*	*Diagnosis*	*Procedure*	*Duration (min)*
1	34	Bilateral hydrosalpinges	BSO	122
2	36	Chronic pelvic pain	TAH + BSO	120
3	40	Stress urinary incontinence	TVH + anterior colporrhaphy	121
4	43	Uterine fibroids/endometriosis	TAH + BSO	130
5	22	Polycystic ovary syndrome	Laparoscopy + right ovarian biopsy	50
6	22	Polycystic ovary syndrome	Laparoscopy + curettage	70
7	28	Polycystic ovary syndrome	Curettage	65
8	31	Polycystic ovary syndrome	Laparoscopy + right ovarian biopsy	60

BSO: Bilateral salpingo-oophorectomy. TAH: Total abdominal hysterectomy. TVH: Total vaginal hysterectomy.

Table II. Mean (±SE) basal concentrations of hormones prior to surgical procedures

Subjects	*LH (mIU/ml)*	*FSH (mIU/ml)*	*PRL (ng/ml)*	*GH (ng/ml)*	*TSH (μU/ml)*
NC (n = 4)	14.66 ± 2.4	11.07 ± 2.47	14.87 ± 1.06	4.1 ± 1.23	1.73 ± 0.19
PCO (n = 4)	42.5 ± 5.71	9.43 ± 0.98	20.84 ± 4.23	4.06 ± 0.6	1.54 ± 0.48
p	<0.01	NS	NS	NS	NS

NC = Normal cycles. PCO = Polycystic ovary syndrome. NS = Not significant.

Two of the women with normal cycles were in the proliferative phase, and the other two were in the luteal phase of their cycles. Dating was based on histologic examination of the endometrium,[14] as well as on determinations of circulating steroid levels (data not shown). In contrast, Patients 5 through 8 were women with polycystic ovary syndrome (PCO) (mean age, 25 years) who underwent a relatively *minor* surgical procedure that lasted approximately 1 hour (61 ± 4 minutes). The diagnosis of PCO was based on characteristic historical features, clinical findings, and biochemical indices.[15] Confirmatory sections of ovarian tissue were available for two of the four patients with PCO; in the other two, the laparoscopic view was considered to be compatible with PCO.

Protocol. All procedures were carried out with the women under general anesthesia. Premedications, administered parenterally 1 hour prior to induction of anesthesia, consisted of a relatively uniform combination of parasympatholytic (atropine sulfate, 0.4 to 0.65 mg), sedative-hypnotic (diazepam, 5 to 10 mg), and narcotic analgesic (morphine sulfate, 5 to 12 mg) agents. Induction was accomplished with thiopental sodium (2 to 5 mg/kg) and succinylcholine chloride (50 to 100 mg). After endotracheal intubation, anesthesia was maintained with N_2O/O_2 mixtures with (4/8) or without (4/8) halothane (0.5 to 1.5%). Muscle relaxation was maintained when needed with succinylcholine chloride and tubocurarine chloride. Anesthesia was terminated by the discontinuation of the inhalation mixture. Both the anesthetic and the surgical procedures were uncomplicated.

Samples of blood (5 ml) were taken at 15-minute intervals for 4 to 8 hours through a three-way stopcock attached to an indwelling venous catheter, which was placed into a large forearm or antecubital vein kept open with an ongoing infusion of normal saline solution.

Analytical methods. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), PRL, GH, and TSH were measured by radioimmunoassay (RIA) as previously described.[16-20] Grouped perioperative data (patients 1-4 and 5-8, respectively) were expressed in terms of the mean (±SE) hormone concentrations. In addition, hormone levels throughout the perioperative period were summed transversely in an attempt to approximate overall hormonal secretory activity. The data have been centered around the time of induction of anesthesia. Extubation served to mark the termination of the procedure. Although the anesthetic and surgical time intervals were not identical, in no case did this interval exceed 10 minutes at either end of the procedure. Consequently, no attempt has been made to separate the effects of anesthesia from those of the surgical procedure per se. Statistical analysis consisted of the Student's t test or the two-factor analysis of variance (ANOVA), as indicated.

Results

Basal hormone concentrations. Basal concentrations (mean ± SE) of five pituitary hormones are depicted in Table II. Baseline levels of hormone represent the mean of three to 15 determinations made prior to the administration of the premedication complex. The mean basal concentration of LH was significantly higher in the PCO group than in the women with normal cycles ($p < 0.01$). In contrast, no significant between-group differences could be detected for the

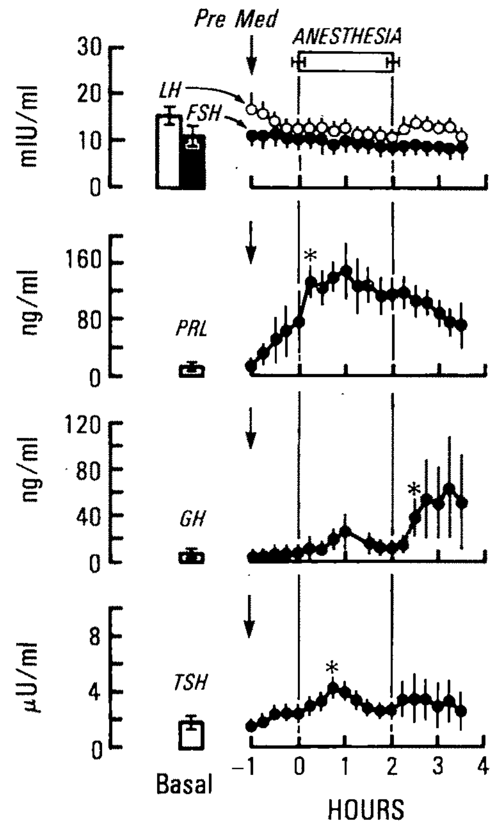

Fig. 1. Mean (±SE) perioperative concentrations of five pituitary hormones in four women with normal cycles who underwent a major surgical procedure. The data have been centered around the time of induction of anesthesia (zero hour). Basal hormone concentrations, depicted as bars (±SEM), represent the mean of three to 15 determinations preceding administration of the premedication complex *(Pre Med)*. *First point at which $p < 0.05$ as compared with mean basal level.

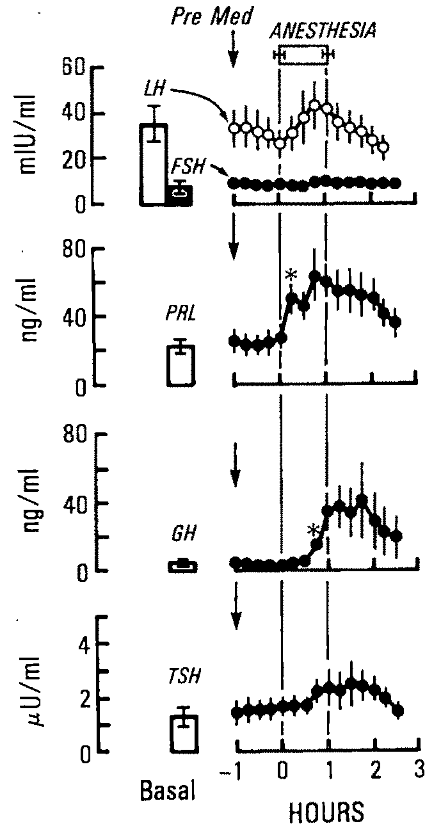

Fig. 2. Mean (±SE) perioperative concentrations of pituitary hormones in four women with polycystic ovary syndrome who underwent a minor surgical procedure. The data have been centered around the time of induction of anesthesia (zero hour). Basal hormone concentrations, depicted as bars (±SEM), represent the mean of three to 15 determinations preceding administration of the premedication complex *(Pre Med)*. *First point at which $p < 0.05$ as compared with mean basal level.

basal mean levels of FSH, PRL, GH, and TSH.

Preoperative interval. The preoperative interval was defined as the time period (60 minutes) between the administration of premedication and the onset of induction of anesthesia. The mean (±SE) preoperative concentrations of five pituitary hormones in the women with normal cycles and in those with PCO are depicted in Figs. 1 and 2, respectively.

In women with normal cycles, the administration of premedication induced a progressive and significant increase ($p < 0.05$) in the overall release of PRL during the preoperative interval (as determined by transverse summation), with attainment of a preinduction peak level of 78 ng/ml. However, this peak value did not achieve statistical significance when compared with baseline, because of substantial fluctuation in individual values. TSH rose from a basal value of 1.7 ± 0.2 μU/ml to a preinduction level of 2.4 ± 0.5 μU/ml. The latter value did not differ significantly from baseline values. Levels of GH and gonadotropins in the women with normal cycles were not significantly different from baseline. Similarly, no significant alterations in the levels of any pituitary hormone could be detected in the individuals with PCO. Between-group comparison of mean hormone levels revealed that the concentrations of PRL (but not of other hormones) changed significantly ($p < 0.01$) in the women with normal cycles, as compared to the women with PCO, during the preoperative interval.

Intraoperative interval. During the intraoperative period, there was a marked rise in PRL levels ($p < 0.05$) in women with normal cycles. The increase in PRL concentrations was prompt, with a mean level of 132 ± 20 ng/ml attained 15 minutes after induction of anesthesia. The elevated PRL levels were maintained throughout the intraoperative period. GH increased from a baseline level of 4 ± 1 ng/ml to a high of 27 ± 14 ng/ml at 1 hour after induction of anesthesia. This increase was not significant because of marked individual variation. However, this temporary elevation was promptly followed by an intraoperative decline to levels comparable to baseline values. The mean TSH level was significantly increased ($p < 0.05$), reaching a peak of 4.3 ± 0.5 μU/ml ($p < 0.05$) 45 minutes after induction of anesthesia. This peak was followed by a gradual decline during the second intraoperative hour. By the conclusion of the surgical procedure, TSH concentrations had fallen to 2.6 ± 0.4 μU/ml (not significant). No significant alterations in circulating concentrations of gonadotropins could be detected.

In PCO subjects this period was marked by significant increments in the mean concentrations of PRL and GH ($p < 0.05$). A significant ($p < 0.05$) increase in PRL levels was observed by 15 minutes after induction of anesthesia, at which time a value of 50 ± 8 ng/ml had been reached. High PRL levels were maintained throughout the intraoperative interval. A significant ($p < 0.05$) increase in the concentration of GH to 14 ± 3 ng/ml was observed 45 minutes after induction of anesthesia. No significant alterations in levels of LH, FSH, and TSH could be discerned, although it appeared that TSH levels were rising during this period (Fig. 2).

When the two groups were compared during the first hour after induction of anesthesia, serum PRL and TSH levels were noticed to be significantly increased ($p < 0.01$) in the women with normal cycles as compared with PCO subjects. No significant differences were observed for the other hormones.

Postoperative interval. The postoperative interval was defined as the time period (90 minutes) after the termination of anesthesia. During this period a progressive fall in PRL levels was observed in both groups. By the conclusion of the sampling period, PRL levels were not significantly elevated in comparison to basal levels. In contrast, GH levels reached their peak in both groups during this period, regardless of the length of the operative procedure. At the conclusion of sampling, GH levels were not significantly elevated as compared with baseline, although variation among subjects was great. No significant differences from baseline could be detected in the levels of gonadotropins or TSH.

Comment

In this comprehensive study of the effects of acute surgical stress on circulating concentrations of pituitary hormones in women, we have observed no change in gonadotropin levels (Figs. 1 and 2). However, we did detect a small but significant ($p < 0.05$) increase in circulating TSH levels in women with normal cycles who underwent a major surgical procedure. We also noticed more profound increments in both PRL and GH levels, which differed in their time course. The numerous variables in such studies of the perioperative period preclude our distinguishing the relative contributions of the various drugs from the stress of the procedure itself.

The lack of any changes in gonadotropin levels that we report is consistent with previous studies in female subjects.[6, 7] In contrast, a significant intraoperative increase in LH in male subjects was previously observed.[7, 21, 22] However, this apparent sex difference remains unexplained. The absence of any significant alteration in gonadotropin levels in our subjects appears to conflict with the findings in animal studies in which acutely administered sedative-hypnotics,[23] opiates,[24] and parasympatholytic[25] agents abolished the preovulatory surge of gonadotropins in the rat. Furthermore, morphine has been found to reduce LH levels in the intact male[26] or castrate female rat.[27] However, the drug dosages employed in those animal studies far exceeded the dosages used during the perioperative period in human beings. Additionally, the rodents were not subjected to a simultaneous surgical procedure.

This study provides detailed evidence of an increase in circulating TSH levels during surgical procedures. This finding cannot be attributed to normal circadian variation, since the intraoperative increase of 150% in the women with normal cycles substantially exceeded the reported maximal diurnal swing of approximately 50%.[28] Furthermore, the increase in TSH levels which we observed occurred over a time interval (0800 to 1100 hours) during which TSH was still declining in normal subjects.[28]

An intraoperative increase in TSH levels has also been observed recently by Adami and associates,[11] who measured TSH in a single sample obtained 30 minutes after induction of anesthesia. A similar tendency, although not statistically significant, was also noticed by Charters and associates.[6] However, other investigators were unable to detect any intraoperative alterations in concentrations of TSH.[6, 8–10, 12, 13] These observations, along with the absence of change in the levels of TSH in subjects with PCO who were undergoing a minor surgical procedure, would suggest that an increased release of TSH is by no means an invariable intraoperative phenomenon, and that it may be related to the degree of surgical stress.

This increase in TSH may be linked to the recently reported intraoperative increments in the concentrations of both free thyroxine (T_4) and free triiodothyronine (T_3).[8, 11, 12] Although the reasons that underlie this augmentation of thyroidal activity remain unclear, a decrease in the binding capacity of thyroid hormone[8] and a direct stress-induced adrenergic stimulation of the thyroid gland have been implicated.[29, 30] However, our finding of a relative intraoperative increase in TSH secretion may provide an alternative explanation.

The increases in both PRL and GH levels that were observed in this study confirm and extend the findings of previous investigations.[1–3, 5, 6] Elevations in PRL were observed prior to or immediately after induction of anesthesia. In contrast, concentrations of GH did not increase significantly until later in the intraoperative period, or not until the postoperative period. Furthermore, PRL, but not GH, levels increased much more in those individuals who were undergoing a major surgical procedure, rather than a minor one.

The increases in PRL and GH are compatible with the effects observed after the administration of opioid compounds to animals[31, 32] and human beings.[33, 34] Although parasympatholytic agents do not appear to affect GH levels in male rats, the administration of cholinergic agonists stimulates the release of GH.[35] The role of cholinergic drugs in the control of PRL release has not been fully clarified.[36, 37]

Together, these data provide evidence for marked changes in the secretion of pituitary hormones during surgical procedures. Whether this pattern of response is unique to acute surgical stress or is more generalized remains to be determined. The effects of each of the various agents used during such procedures will need to be elucidated, as well, in future studies.

Materials for the radioimmunoassay of LH, FSH, PRL, GH, and TSH were kindly provided by the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland. The assistance of Ms. Del Austin in the typing of this manuscript is greatly appreciated.

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Sonar cephalometry in twin pregnancy: Discordancy of the biparietal diameter after 28 weeks' gestation

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Sonar measured biparietal diameter (BPD) differences of twin pairs were examined in 123 twin pregnancies at or beyond 28 weeks' gestation. Among 117 liveborn sets, the risk of a twin infant being small for gestational age was threefold greater when paired BPD differences were 5 mm or more compared to 4 mm or less. The incidence of fetal death increased from 2.7% for twin pairs with 0 to 6 mm BPD differences to 20% when the difference was 7 mm or more. Sonar cephalometry may be helpful in the antepartum evaluation of twin pregnancies, although detection of BPD discordancy does not preclude normal twin outcome. (AM. J. OBSTET. GYNECOL. 138:615, 1980.)

DETECTION of fetal jeopardy in singleton pregnancy has been the focus of extensive investigation by means of a variety of endocrine, electronic, and ultrasonic techniques. Although the twin fetus is comparatively in greater danger,[1] antepartum tests in use for singletons have yielded confusing results in the presence of two fetuses. Previous studies with sonar cephalometry in twin gestations have encouragingly indicated that the discovery of large twin pair biparietal diameter (BPD) differences may improve ascertainment of fetal jeopardy.[2-6] We investigated 123 twin gestations that underwent 353 sonar examinations to determine the risk of fetal growth retardation and fetal death in relation to twin pair BPD differences observed within 4 weeks of delivery. Additionally, this article reports the frequency distribution of paired twin BPD differences, the relationship of these differences to birth-weight dissimilarity, and the incidence of BPD discordancy during three periods of twin gestation.

Material and methods

Between January 1, 1972, and December 31, 1977, 294 women with twin gestations underwent sonographic examination in the Ultrasound Laboratory of the Department of Obstetrics and Gynecology at The University of Texas Southwestern Medical School. The method of sonar twin cephalometry has been described previously.[7] For this study of BPD discordancy among twin fetuses, 123 women, hospitalized for most of the third trimester on the Parkland Memorial Hospital High-Risk Pregnancy Unit, were selected. Although some of the pregnancies studied were included in a previous report,[7] the present study population differs significantly because of different selection criteria.

The selection criteria used in this study are as follows: (1) A minimum of one sonar examination was made at or beyond 28 weeks' gestation and within 4 weeks of delivery. (2) Both fetuses were alive during cephalometry. (3) Separate BPD measurements were obtained during at least one sonar examination. (4) Gestational age based upon menstrual history was in accordance with sonographic estimates.

A total of 353 sonar examinations was performed on the pregnancies selected. Maximum differences in BPD between fetuses of each twin set measured during the 4 weeks prior to delivery were grouped according to their magnitude into four categories: 0 to 2 mm, 3 to

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Sponsored by the Society for Gynecologic Investigation.

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Table I. Frequency distribution of BPD differences within sets of 123 twin gestations

BPD difference (mm)	*Number of gestations*	*Percent*
0-2	56	45.5
3-4	31	25.2
5-6	21	17.1
≥7	15	12.2
Total	123	100.0

Table II. Grouped twin pair birth-weight differences in relation to BPD differences

Birth-weight difference (%)*	*No. of pairs (%)*	*Mean BPD differences (mm)*	*Range of BPD differences (mm)*
0-5	31 (26.5)	3.1	0-11
6-10	33 (28.2)	2.7	0.7
11-15	31 (26.5)	3.1	0.7
16-20	12 (10.3)	4.3	0-14
21-25	4 (3.4)	6.2	2-7
>25	6 (5.1)	8.2	0-16

*Using larger infant as 100% and including only liveborn pairs.

Table III. Risk of fetal growth retardation in relation to grouped BPD differences

BPD difference (mm)	*No. of pregnancies*	*No. of twin pairs with one SGA infant* (%)*
0-2	55	3 (5.5)
3-4	30	3 (10)
5-6	20	5 (25)
≥7	12	2 (16.7)

*Among liveborn twin pairs when one infant of a pair was small for gestational age.

4 mm, 5 to 6 mm, and 7 mm or greater. For each liveborn twin pair, the percentage difference in birth weight was calculated, with the larger twin used as 100%. A neonate was classified as small for gestational age (SGA) if its birth weight was more than one standard deviation below the mean for twins reported by Hendricks.[8]

Statistical analysis of data was performed using chi-square contingency table analysis or Fisher's exact probability test.

Results

The 123 women whose pregnancies are described in this report were predominantly black (63%); 22% were white; and 15% were of Hispanic origin. The mean gestational age at delivery was 36.9 weeks, with a range of 29 to 41 weeks. Varying degrees of maternal hypertension complicated 54 of the pregnancies studied.

The infant pairs delivered were of different sex in 31 instances, and approximately 18% of liveborn twins were small for gestational age. Of these, both members of a twin pair were SGA in 15 pregnancies, whereas only one member of a pair was SGA in 13.

In Table I, the frequency distribution of BPD differences within twin sets is grouped into four categories. The BPDs of twin pairs were within 4 mm of each other in approximately 71% of cases. Greater degrees of BPD difference were infrequent, with only 12% demonstrating 7 mm or more dissimilarity. Grouping of twin pair birth-weight differences into six categories (Table II) of increasing magnitude revealed that the incidence of a particular birth-weight difference did not change until weight variation of a twin pair exceeded 15%. Similarly, the mean BPD difference observed for each birth-weight category did not increase until pairs differed by more than 15%. Thus, 95 (77%) twin pairs had birth weights within 15% of each other and a mean BPD difference of less than 4 mm. In contrast, the mean BPD difference observed for twin pairs with more than 15% birth-weight differences increased progressively from 4.3 mm to 8.2 mm. When the incidence of paired birth-weight differences exceeding 15% was examined in relation to grouped BPD differences, the incidence increased progressively and became statistically significant when BPD differences were 5 mm or more, compared to 4 mm or less ($\chi^2 = 7.00$, $p < 0.01$), that is, only 11 (13%) of 85 twin pairs with BPD differences 4 mm or less had >15% birth-weight differences compared to 11 (35%) of 32 pairs for whom BPD differences were 5 mm or more. Although the trend of these data suggests a correlation between sonar measured head-size differences and birth-weight differences, the range of variation of the mean BPD difference shown in Table II is so great that prediction of body-size differences in an individual twin pair is imprecise.

The number of twin pairs in which *one* of the infants was growth retarded increased as biparietal differences increased (Table III). When the BPD difference was 5 mm or more, compared to 4 mm or less, the number of pairs with one SGA infant increased significantly from 7.1% to 21.7% ($\chi^2 = 5.17$, $p < 0.05$). There was no correlation between BPD differences and delivery of twin pairs when *both* infants were SGA.

Three neonates died of complications of prematurity and one neonate died of dysplastic kidney disease. There were seven fetal deaths, two of which occurred in one pregnancy. The pregnancies complicated by fetal death are summarized in Table IV. When twin pairs were grouped according to their maximum BPD difference in the third trimester, as shown in Table V, the risk that a twin pregnancy would be complicated by fetal death increased as sonar measured biparietal dif-

Table IV. Case summaries of fetal deaths

Case No.	Maximum BPD (mm) difference	Pregnancy complications	Birth weight (grams) Sex/Presentation A	Birth weight (grams) Sex/Presentation B	Fetal age at death (wk)	Fetal membrane description
1	3	Previous stillbirth	2,830 Female Cephalic	(2,700) Female Breech	39	Diamnionic Monochorionic
2	13	Pregnancy-induced hypertension	2,863 Male Cephalic	(1,389) Male Breech	36	Diamnionic Monochorionic
3	0	None	(2,375) Female	(2,500) Female	40	Diamnionic Monochorionic
4	17	Pregnancy-induced and essential hypertension	1,875 Male Cephalic	(905) Male Cephalic	35	Diamnionic Dichorionic
5	7	None	(1,100) Female Cephalic	2,330 Female Transverse	33	Diamnionic Dichorionic
6	6	Hydramnios	1,890 Male Cephalic	(1,240) Male Breech	35	Diamnionic Monochorionic

( ) = Fetal death.

ferences increased. In pregnancies with 7 mm or more BPD difference, the risk of fetal death was approximately 20% compared to a 2% to 5% rate with lesser degrees of BPD difference. The difference between 7 mm BPD dissimilarity and lesser degrees was statistically significant (Fisher's exact probability, 0.024).

When the incidence of biparietal differences exceeding 4 mm was analyzed during three periods of gestation (Table VI), differences of this magnitude increased significantly as gestation advanced ($\chi^2 = 9.99$, $p < 0.01$). There were two pregnancies with more than 4 mm difference while less than 24 weeks' gestation. In one case the BPD difference was 5 mm at 20 weeks' gestation, and in the other it was 6 mm, also at 20 weeks. In both cases these BPD differences were not found during subsequent serial cephalometry. The case with a 6-mm BPD difference resulted in abruptio placentae at 33 weeks' gestation, with the infants weighing 1,900 and 1,605 grams. Delivery of the pregnancy with a 5-mm BPD difference at 20 weeks' gestation was at 39 weeks, and the infants weighed 2,497 and 2,128 grams. The smaller infant died of renal failure, and dysplastic kidneys were found at autopsy.

Comment

Several reports have confirmed that twin birthweights after 30 weeks' gestation are less than those of singletons of the same gestational age.[8-11] Other investigators have found SGA infants to be prevalent among twins[12, 13] and, in two reports, have found higher mortality rates for these infants.[8, 14] Accelerated postnatal growth,[15] coupled with the low birth weight of twin infants, suggests in utero malnutrition, probably a result of limited maternal potential to nurture multiple fetuses.[16]

Although it is clear that twin infants are undergrown at birth in comparison to singletons, it is also apparent that some twin siblings are unequally nourished during gestation and that the smaller infant has a poorer prognosis. This is borne out by the observation that, in twin gestations complicated by stillbirth, it is commonly the smaller infant who succumbed. During the postnatal period, the smaller of discordant twins has been reported to suffer retarded growth and development,[17-20] as well as an increased incidence of hypo-

Table V. Antepartum death of twin fetuses among pairs with varying BPD differences

BPD difference (mm)	No. of pregnancies	Pregnancies with fetal death (%)
0-2	56	1 (1.7)
3-4	31	1 (3.2)
5-6	21	1 (4.7)
≥7	15	3 (20.0)
Total	123	6 (2.4)

Table VI. BPD discordancy within sets of twins in three periods of gestation

Initial sonogram	No. of gestations	No. of twin pairs with a BPD difference of ±5 mm
<24 wk	29	2 (7.0%)
24-28 wk	48	6 (12.5%)
>28 wk	123	36 (29.3%)

glycemia.[21] These findings have been attributed to asymmetrical intrauterine nutrition. Similarly, Naeye[22] linked antenatal malnutrition to his findings of reduced organ cytoplasmic mass in discordant donor twins affected by the placental transfusion syndrome.

Most simplistically, unequal growth in twins, with the larger twin used as an internal standard, could serve to identify fetal jeopardy in a twin gestation. Unfortunately, clinical evaluation of twin fetal size, or co-twin size differences, is seldom accurate because physical examination by palpation of the twin pregnancy is very difficult. Previous reports have indicated that use of serial sonar cephalometry to detect BPD discordancy may be helpful in the identification of pregnancies destined to suffer fetal growth retardation or fetal death.[2-6] Biparietal diameter differences of 6 mm or more were generally indicative of a poor fetal outcome. Our results statistically substantiate these findings.

The great majority (71%) of twin pairs were within 4 mm of each other after 28 weeks' gestation, a difference that was not associated with any adverse outcome. When BPD differences exceeded 4 mm, correlations were found to birth-weight differences of a pair exceeding 15% and growth retardation in one member of the pair. Importantly, the incidence of fetal death increased significantly when BPD differences were 7 mm or more. Although twin pairs with BPD differences of 5 mm or more were statistically separated from those with 4 mm or less, only 20% to 30% of twin pairs with this degree of BPD discordancy were found to have significant birth-weight differences, growth retardation of one fetus, or fetal death. This implies a significant false positive rate if BPD difference is used as the sole method of detecting fetal jeopardy in twin gestations. For example, although 15 gestations demonstrated BPD differences of 7 mm or more in the third trimester, only three were complicated by fetal death. Therefore, 80% of the twin pregnancies with large BPD differences did not suffer a fetal death.

There are two likely explanations for the failure of sonar cephalometry to more accurately predict significant twin pair size differences. First, twin presentations are often unstable, thus preventing reliable BPD measurement of at least one fetus. Because of this difficulty, many pregnancies were excluded from our study, since the sonographer was not certain that reliable BPD measurements had been obtained for each fetus. The second explanation is based on the widely recognized poor correlation between the biparietal diameter and birth-weight. Measurement of the fetal abdominal circumference in addition to the BPD may be a helpful adjunct in detection of twin birth-weight discordancy. This is currently under investigation.

An interesting observation was the increased incidence of BPD discordancy as the gestation advanced. BPD differences of 5 mm or more were more frequent in pregnancies examined after 28 weeks' gestation, as compared to pregnancies studied before 24 weeks. Other investigators have found tapering of twin fetal growth late in pregnancy,[8-11] and our finding of more frequent BPD dissimilarity in late twin pregnancy is further evidence that the third trimester of twin gestation is a critical period in the growth of twin fetuses.

Consideration must be given to the possibility that discordancy in early pregnancy has a different prognosis than when it develops in the third trimester. BPD discordancy was noted at 25 weeks' gestation in one case with subsequent fetal death and may indicate that discordancy early in gestation is ominous. A second similar case managed after the completion of this study supports this observation. However, our data do not permit accurate appraisal of fetal risk according to when in gestation discordancy was present, since only 29 patients underwent cephalometry before 24 weeks' gestation. In this group, there were two instances of significant BPD discordancy, and the outcome was poor in both pregnancies but not clearly related to discordancy in either case. The latter is especially true since both cases were not found to have BPD discordancy during subsequent cephalometry. This finding was probably due to technical difficulties encountered in measuring twin BPDs rather than a resolution of disparate growth as gestation advanced.

In summary, we believe that serial sonar cephalometry is an important method of evaluating fetal prognosis in twin pregnancies. Large BPD differences of a twin pair provide a clue that the pregnancy is at risk for fetal growth retardation and fetal death. The clinician must keep in mind that BPD discordancy is a less than ideal predictor of outcome, since accuracy is poor. As a result of our findings, we have been prompted to consider early intervention when there are large BPD differences, but to undertake such intervention cautiously and only when fetal pulmonary maturity is established.

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The use of thyrotropin-releasing hormone in distinguishing prolactin-secreting pituitary adenoma

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In order to differentiate between a prolactin-secreting adenoma and a nonneoplastic etiology for galactorrhea, a single stimulatory dose of 500 μg of thyrotropin-releasing hormone (TRH) was employed to assess the prolactin (PRL) response of 50 women. The results were compared to those obtained in 19 control subjects (10 with subsequent surgical removal of a PRL-secreting adenoma and nine women stimulated in the early follicular phase). All patients were grouped according to normal or elevated serum PRL, and normal, equivocal, or abnormal findings by tomography of the sella turcica. Patients with a normal baseline PRL had a normal PRL response to TRH of at least 200% above baseline. This normal response was independent of findings by tomography of the sella turcica. A highly significant difference in response ($p < 0.001$) was illustrated by all patients with elevated PRL and abnormal tomograms, and in some patients with elevated PRL and normal or equivocal tomograms. These responses were identical to those of the control patients who had histologic evidence of a PRL-secreting adenoma. The findings indicate that a PRL-secreting adenoma in patients with galactorrhea can be identified on the basis of the PRL response to TRH stimulation. (AM. J. OBSTET. GYNECOL. 138:620, 1980.)

VARIOUS STIMULATORY as well as inhibitory tests of the hypothalamic pituitary axis have been purported to delineate whether patients with galactorrhea have a neoplastic cause for it.[1-5] The results of these tests (L-dopa, chlorpromazine, gonadotropin-releasing hormone) have been reported to be variable and, therefore, cannot be predictive.[6] Although the administration of thyrotropin-releasing hormone (TRH) may be beneficial in identifying prolactin-secreting pituitary adenoma,[1, 2] the findings have not been consistent.[7] Because of the availability of TRH and the minimum side effects found with the use of it, a prospective analysis of TRH stimulation in patients with galactorrhea was undertaken. In order to qualify the prolactin (PRL) response to TRH stimulation as either normal or abnormal, two separate control groups were investigated. In normally cycling women, TRH stimulation was performed in the early follicular phase of the cycle, and a 95% confidence interval for a normal PRL response was generated. TRH stimulation was also performed in patients with presumptive evidence of a PRL-secreting adenoma. Transsphenoidal exploration was subsequently performed in the latter group of patients, and all were found to have a PRL-secreting pituitary adenoma. With these two groups of controls, a prospective study was undertaken to determine the efficacy of TRH stimulation in identifying PRL secreting adenomas.

Material and methods

Sixty-nine subjects participated in this study. Of these, 50 patients, ages 18 to 36 years, with galactorrhea and normal or abnormal menses were divided into groups on the basis of hypocycloidal tomography of the sella turcica and baseline serum PRL levels (Table I). The other 19 subjects comprised two groups of controls: nine normally cycling women were tested in the early follicular phase of their menstrual cycle and were used as a representative population for a normal test response; and 10 women with abnormal

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Presented at the Twenty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 21-24, 1979.

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**Recipient of Jeanne B. Kempner Fellowship Award.*

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Table I. Clinical and laboratory findings in patients with galactorrhea

			Menses		*TRH test*	
No. of patients	*Hypocycloidal tomography*	*PRL*	*Normal*	*Oligomenorrhea-amenorrhea*	*Normal*	*Blunted*
11	Normal	Normal	8	3	11	–
9	Normal	Elevated	4	5	6	3
4	Equivocal	Normal	4	–	4	–
9	Equivocal	Elevated	3	6	2	7
8	Abnormal	Normal	3	5	8	–
9	Abnormal	Elevated	3	6	–	9
9*	–	Normal	9	–	9	–
10†	Abnormal	Elevated	–	10	–	10

*Early follicular phase controls.

†Controls with surgically proved adenoma.

sella tomograms and elevated PRL were stimulated with TRH. Because subsequent surgical exploration in these patients revealed a PRL-secreting pituitary adenoma, the preoperative TRH tests were used as a representative pattern of PRL response for patients harboring an adenoma.

All study subjects (50) and controls (19) had a single TRH stimulation test. A No. 21 gauge butterfly needle was inserted into an arm vein and kept open with a heparin solution. A baseline sample of blood was obtained prior to injection of a single intravenous bolus of 500 μg of TRH. Thereafter, samples of blood were obtained at 20, 30, and 60 minutes. Plasma was separated by centrifugation and stored at −20° C, until assayed. Plasma levels of PRL and TSH were measured by previously reported radioimmunoassay procedures.[8, 9] Baseline estrogen values were determined for all patients.[8] PRL response was calculated as a percentage change from baseline as well as the area under the curve.[10] The Mann-Whitney "U" test was used for significance.[11]

Results

Controls. The nine cycling women had a normal baseline plasma PRL value (6.5 to 12.8 ng/ml) and a normal plasma thyroid-stimulating-hormone (TSH) (0.9-2.6 μU/ml). After injection of TRH, there was an initial significant increase in PRL at 20 minutes (26 to 150 ng/ml, 95% confidence limit) and in TSH (8 to 45 μU/ml, 95% confidence limit) at 30 minutes, declining thereafter (Fig. 1). On the basis of these results, the 95% confidence interval was determined as a percentage change from baseline. The confidence limits ranged from 200 to 1,500%. In contrast, patients who were proved to have adenoma and an elevated baseline plasma PRL (55 to 1,800 ng/ml) and a normal baseline TSH had no significant PRL response to TRH administration (Fig. 1). The TSH response was similar to that seen in the early follicular phase.

Patients. Twenty-three patients with normal baseline PRL (<20 ng/ml) and normal, equivocal, or abnormal findings by tomography had a normal PRL response to TRH stimulation (Fig. 2). Release of TSH was normal in all but one patient. This patient had an elevated baseline TSH (5.7 μU/ml) and an exaggerated response (68 μU/ml) to the administration of TRH, indicative of hypothyroidism.

Twenty-seven patients with elevated baseline prolactin (21 to 397 mg/ml) had a variable PRL response to TRH stimulation (Fig. 3). Among the eight patients with normal sella tomograms and elevated baseline prolactin (21 to 260 ng/ml), five had a normal PRL response to TRH. The other three patients had no PRL response, and thus were similar to the patients with proved adenoma. Baseline PRL levels in the three nonresponders were 45, 176, and 260 ng/ml. TSH response in these patients was normal.

Among the nine patients who had equivocal tomograms and elevated PRL baseline (22 to 176 ng/ml), two had a normal PRL response to TRH (Fig. 3). The other seven patients had an abnormal PRL response to TRH stimulation. Three patients had a blunted response (<200% increase) whereas four had a flat response similar to that seen in patients with adenomas. PRL baselines in these seven patients ranged from 29 to 176 ng/ml. TSH response was normal in all patients, with the exception of one who had an elevated baseline TSH of 12.0 μU/ml. This patient had an exaggerated TSH response (103.2 μU/ml) consistent with hypothyroidism.

All nine patients with abnormal sella tomograms had elevated baseline PRLs ranging from 31.2 to 402 ng/ml and a flat PRL response similar to that of patients with proved adenoma (Fig. 3). TSH response was normal in this group of patients.

When the responses in all patients were compared to those in the normal controls, as to percentage change from baseline, a delineation of normal and abnormal

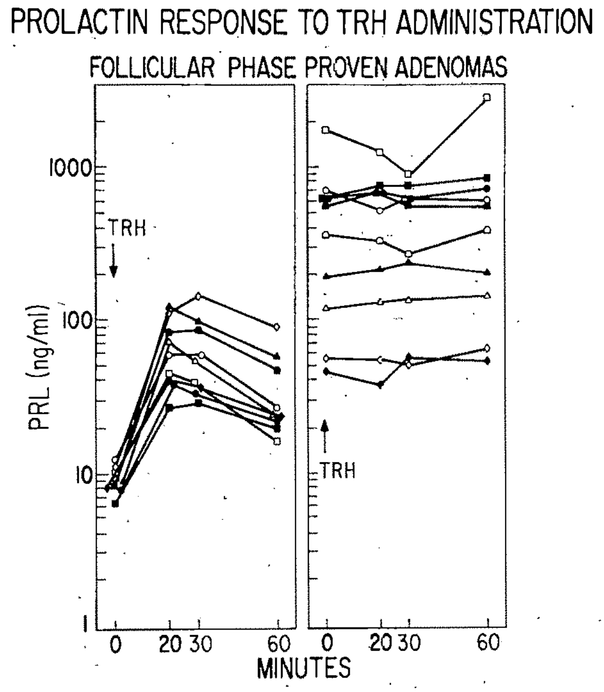

Fig. 1. Plasma PRL concentration after TRH stimulation in early follicular phase subjects and patients with proved adenoma.

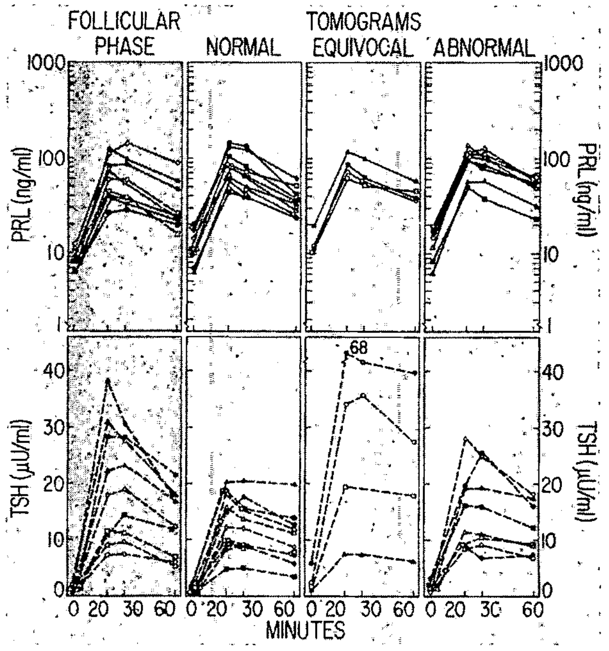

Fig. 2. Plasma PRL and TSH concentration after TRH stimulation in patients with normal PRL baseline and normal, equivocal, or abnormal tomograms.

responses in the various groups was evident (Fig. 4). If a 200% change from baseline was not obtained, the response was considered to be abnormal. Moreover, similar results were obtained when PRL response was calculated as total area under the curve (Figs. 5 and 6). Those patients with a normal PRL baseline had a net increase similar to that in normal controls and significantly different from that in patients with proved adenoma ($P < 0.001$) (Fig. 5).

If the patients with elevated baseline PRL responded to TRH, the net increase was similar to that of normal controls. Those patients with a blunted response had a net PRL increase similar to that of patients with proved adenoma. The net PRL increase in nonresponders was significantly different from that in normal control subjects ($P < 0.001$) (Fig. 6). The patients who had a normal PRL response to TRH had a mean baseline estrogen of 122.7 ± 18.1 pg/ml, whereas the patients who showed no PRL response after TRH stimulation had a mean baseline estrogen of 82.1 ± 18.7 pg/ml. This difference was not statistically significant.

Comment

The findings in this study demonstrated that the response of prolactin to the administration of TRH may be a sensitive indicator of pituitary responsiveness in the presence or absence of a PRL-secreting pituitary adenoma. In previous reports, it was stated that patients with a PRL-secreting pituitary adenoma may have lost their ability to respond to the administration of TRH, whereas L-dopa or bromocriptine maintained their inhibitory effect.[1, 2, 6] This is demonstrated by the group of patients with a histologically proved adenoma who were used as controls in this study. Patients with an elevated baseline prolactin and abnormal findings by tomogram had a similar blunted PRL response to TRH, which strongly suggested that those patients had a PRL-secreting pituitary adenoma. In fact, two of these patients subsequently underwent transsphenoidal sella exploration and were found to have a PRL-secreting pituitary adenoma.

Interpretation of an equivocal tomogram presents a special problem. It may represent a normal variation or a very early change due to the presence of a microadenoma. On the basis of the findings in this study, it seems possible to differentiate patients with an equivocal tomogram who may have a tumor (blunted PRL response) from those who may have a sella turcica within the limits of normality (normal PRL response). Furthermore, patients with normal tomograms and elevated baseline PRL may also have an abnormal PRL response to TRH. The TRH test may be a more sensitive early indicator of a pituitary adenoma. It appears that dysfunction of the lactotrophs may precede defi-

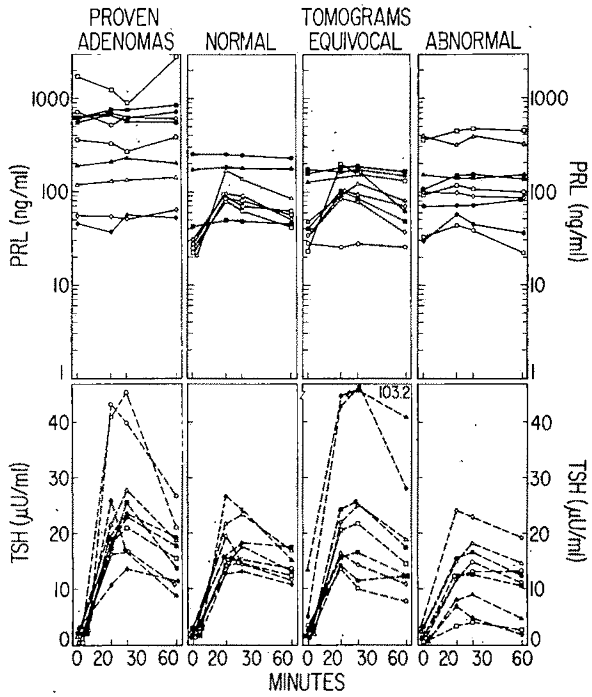

Fig. 3. Plasma PRL and TSH concentration after TRH stimulation in patients with elevated PRL baseline and normal, equivocal, or abnormal tomograms.

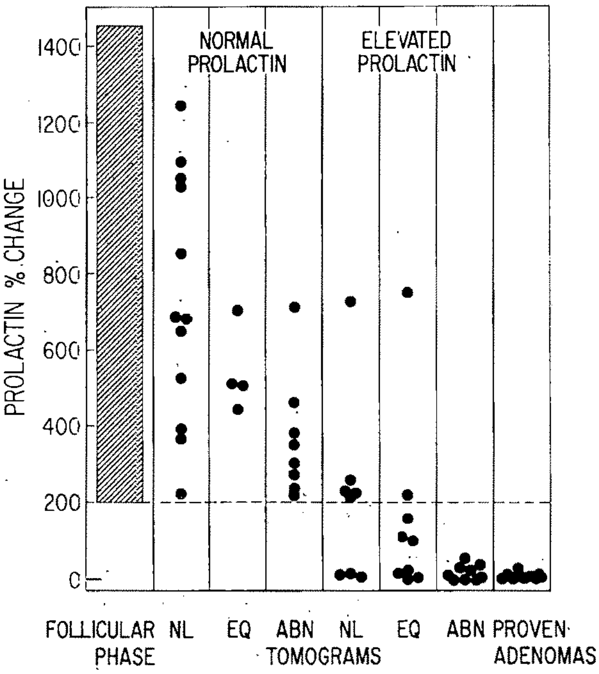

Fig. 4. PRL response in all subjects after TRH stimulation compared as percentage change from baseline.

nite radiographic changes. This is supported by the finding of three patients with blunted PRL responses and normal tomograms, as well as seven other patients with elevated PRL and equivocal tomograms. However, no definitive statement can be made without surgical exploration of the three patients with normal tomograms and the seven patients with equivocal tomograms. As previously reported, the TSH response to TRH is within normal range in most of these patients, with the exception of those with primary hypothyroidism who had an exaggerated response.[11]

The TRH test seemed to be less meaningful in patients with normal baseline PRL, since the response to TRH stimulation was normal, irrespective of the findings by tomography. The normal PRL response to TRH in patients with abnormal tomograms and normal baseline PRL is difficult to explain. In these patients, the neuroradiologist was unable to differentiate between subarachnoid herniation, craniopharyngioma, and PRL-secreting pituitary microadenoma at the time of hypocycloidal tomography. In view of the fact that these patients had a normal PRL response to TRH stimulation, and a normal baseline PRL, it is likely that they did not have a PRL-secreting adenoma as the etiologic factor for galactorrhea.

Currently, this group of patients is being evaluated to determine the exact cause of the galactorrhea. Four of these patients have undergone computerized axial tomography with metrizomide contrast infusion. Three patients were found to have an empty sella, and the fourth patient had a craniopharyngioma. These findings are in agreement with those of a recent report that dealt with the empty sella syndrome.[13] Currently, one other patient in this group is being followed with a spontaneous intrauterine pregnancy. The other three patients are presently under investigation.

There are controversial reports of PRL response to TRH administration in subjects with normal or low levels of serum estradiol.[14-17] In addition, this study demonstrates that no pattern of menstrual abnormality could be correlated to the PRL response (Table I). Moreover, that no difference was seen in mean estrogen levels between the responders and nonresponders adds even more support to the fact that circulating estrogen levels have little, if any, influence on PRL response to TRH stimulation.

It was recently reported that patients with an elevated PRL and a normal cone view of the sella turcica are the only patients with galactorrhea who need hypocycloidal tomography to rule out an abnormality of the sella turcica.[18] This was suggested because seven of 87 patients were found to have an abnormal tomogram in the presence of a normal cone view and an elevated baseline PRL. On the basis of the findings in

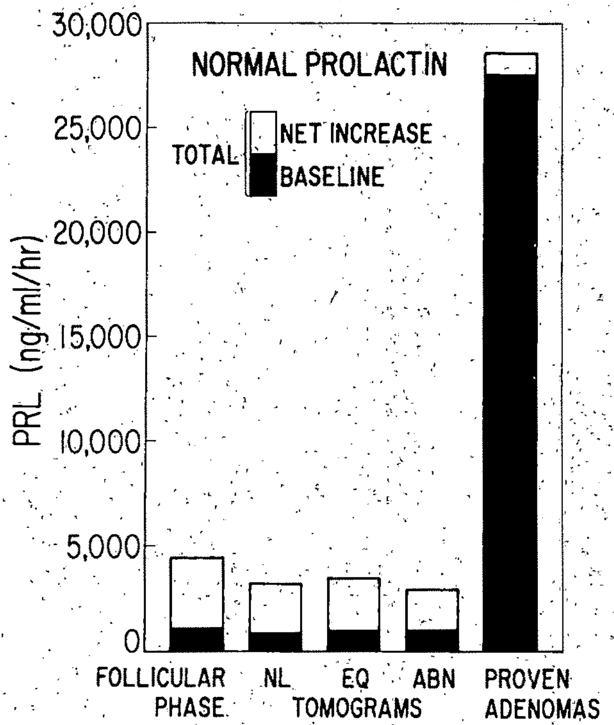

Fig. 5. PRL response in patients with normal PRL baseline as determined by the total area under the curve.

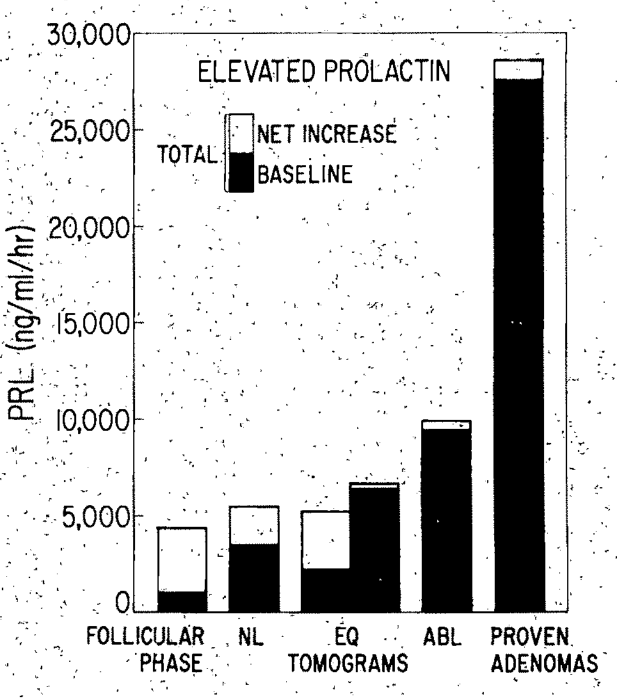

Fig. 6. PRL response in patients with elevated PRL baseline as determined by the total area under the curve.

this study, it is conceivable that with the addition of the TRH stimulation test it will be possible to identify those patients in whom sella tomography is necessary for a diagnosis. If this is correct, only patients with galactorrhea and an elevated baseline prolactin who have an abnormal PRL response to TRH may benefit from hypocycloidal tomography.

The TRH test is simple and can be used as an office procedure. By obtaining a baseline sample of blood before administration of TRH and then a second sample of blood 20 to 30 minutes later, one can easily calculate a normal PRL response as a percentage change from baseline.

A simple calculation is used to determine the percentage change from baseline. The baseline value is subtracted from the value at 30 minutes, and the difference is then divided by the baseline, which is multiplied by 100. A normal response of 200% change would be interpreted as an increase from a baseline PRL of 10 ng/ml to a level of 30 ng/ml at 30 minutes. In other words, a PRL response that triples the baseline value is considered to be a normal response.

It is possible that the TRH stimulation test can be used to follow patients with elevated baseline PRL and an initial normal PRL response to TRH. As long as the PRL response to TRH is normal, repeat radiographic studies may not need to be performed.

In conclusion, TRH seems to be a useful clinical test for the differentiation of PRL-secreting pituitary adenoma in patients with galactorrhea and an elevated baseline serum PRL.

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Hormone production during pregnancy in the primigravid patient

II. Plasma levels of deoxycorticosterone throughout pregnancy of normal women and women who developed pregnancy-induced hypertension

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The plasma concentration of deoxycorticosterone (DOC) was determined serially in a large group of primigravid women from 10 weeks' gestation to term. The plasma level of DOC in women whose pregnancies were uncomplicated (n = 44) was 234 ± 33 pg/ml (mean ± SE), at 10 to 14 weeks' gestation, a level two times that of nonpregnant subjects. The plasma level of DOC in these women rose to 778 ± 65 pg/ml at 23 to 26 weeks' gestation, and at term (39 to 42 weeks) was 1,309 ± 155 pg/ml. The plasma levels of DOC in women (n = 31) who eventually developed pregnancy-induced hypertension (PIH) were similar to those in the women who remained normal at all stages of pregnancy. We also found that, in women with normal pregnancies as well as in women destined to develop PIH, the plasma concentration of DOC fluctuated in a manner parallel to that of progesterone throughout gestation; however, changes in the plasma level of DOC did not mirror those of cortisol. These data suggest that excessive plasma levels of DOC are not necessarily associated with the development of PIH. These data also support the view that, in pregnant women, a fraction of circulating DOC may arise via extra-adrenal 21-hydroxylation of progesterone rather than through adrenal secretion. (AM. J. OBSTET. GYNECOL. 138:626, 1980.)

THE PLASMA concentrations of a number of steroid hormones are increased strikingly during the course of human pregnancy. The origin, production rates, and metabolism of several steroid hormones in pregnant women have been established, but information concerning the origin of deoxycorticosterone (DOC), a potent mineralocorticosteroid, is limited. Brown and associates[1] found that the plasma levels of DOC were significantly higher in pregnant women near term than in nonpregnant women. Nolten and associates[2] studied seven patients sequentially during pregnancy and found that plasma levels of DOC during the first trimester were increased 2.5-fold over the levels in nonpregnant women. They also found that the levels of DOC increased further during gestation with the levels attained during the third trimester of pregnancy being sixfold greater than those in nonpregnant subjects. In view of this increase, it is surprising that most women are able to maintain a normal salt and water balance as well as blood pressure during the latter stages of pregnancy.

The reported increases in the plasma concentrations

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Supported in part by National Institutes of Health Grants 5-P50-HD11149 and 5-RO1-HD008360.

Presented at the Twenty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 21-24, 1979.

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Table I. Profile of primigravid subjects

Patient category	*No. of subjects*	*Group age**	*Ethnic origin*		
			Caucasian	*Black*	*Latin American*
Normotensive	44	18.5 ± 2.06	12	29	3
Pregnancy-induced hypertension	31	18.1 ± 3.63	6	22	3

*Years of age at delivery, mean ± SD.

of DOC during pregnancy make it seem reasonable to ascertain whether an alteration(s) in DOC metabolism might in some manner be related to the development of pregnancy-induced hypertension (PIH). Whereas data are available on the levels of DOC in the plasma of women after the development of hypertension, plasma concentrations of DOC prior to the development of symptoms are undocumented. It has been proposed that, in women who develop PIH, the development of hypertension and associated symptoms may be related to an excessive production of mineralocorticosteroids.[3] However, Brown and associates[1] found that the plasma levels of DOC in seven women with PIH during the latter stages of the third trimester did not differ from those of normotensive women during the third trimester. Contrasting results were obtained by Weir and associates,[4] who reported that plasma concentrations of DOC in women with hypertension and proteinuria during the third trimester were significantly lower than those of normotensive pregnant women. However, the values in both of these studies were obtained in women only after the development of hypertension.

The present investigation was undertaken to define more fully the changes in plasma levels of DOC throughout normal primigravid pregnancy. A second objective was to ascertain whether alterations in the production of DOC, as reflected by its level in plasma, might precede the development of PIH in primigravid women. Finally, since plasma levels of DOC respond poorly to ACTH stimulation or dexamethasone suppression in pregnant women during the third trimester,[1, 2, 5] and since we have found that circulating progesterone is metabolized to DOC in human beings,[6, 7] we also sought to ascertain whether plasma levels of DOC parallel those of progesterone during pregnancy in primigravid women.

Material and methods

Subjects. Volunteers for this study were chosen from the outpatient clinic population of the Department of Obstetrics and Gynecology of Parkland Memorial Hospital, Dallas, Texas. A large group of primigravid women were followed from as early as 10 weeks' gestation through term. Samples of blood were obtained at approximately biweekly intervals while the patients were resting in the lateral recumbent position. None of the primigravid women included in this study had a prior history of hypertension or diabetes. Samples of plasma obtained from 44 women whose pregnancies remained normal (total number of samples of plasma = 380) and from 31 women who developed PIH (total number of samples of plasma = 265) were chosen for analysis. The diagnosis of PIH and the rating of the severity of the disease were made independently by four obstetricians who reviewed the records of each patient retrospectively, without knowledge of hormonal findings. The severity of PIH was defined as follows: mild PIH = an increase of 20 mm Hg in diastolic blood pressure over the level at midpregnancy and at least a 90 mm Hg diastolic blood pressure on two occasions 6 hours apart; moderate PIH = persistent diastolic blood pressure of more than 100 mm Hg; severe PIH = persistent diastolic blood pressure of more than 110 mm Hg in the presence or absence of albuminuria, or an onset of hypertension before the thirty-fourth gestational week. There were 20 women with mild PIH, five women with moderate PIH, and six women with severe PIH. The age and race of these subjects are listed in Table I.

Measurement of plasma concentration of DOC. For the measurement of DOC, plasma samples were mixed with 1,000 cpm of [1,2-^{3}H(N)]deoxycorticosterone, 46.8 Ci/mmol, and extracted with 10 volumes of diethyl ether. The organic phase was removed and evaporated, and the residue was chromatographed on celite-ethylene glycol (1:0.75, w/v) columns. The columns were eluted sequentially with 5 ml each of isooctane and isooctane:ethyl acetate (100:7.5, v/v). The last 4 ml of the elute, after the second solvent system was applied, contained DOC, which was assayed by radioimmunoassay. The antiserum[8] used in the assay of DOC was generously supplied by Dr. C. E. Gomez-Sanchez. The cross reaction of the antiserum with the following steroids was less than 1%: cortisol, pregnenolone 17α-hydroxyprogesterone, 11-dehydrocorticosterone, corticosterone, testosterone, 18-hydroxy-DOC,

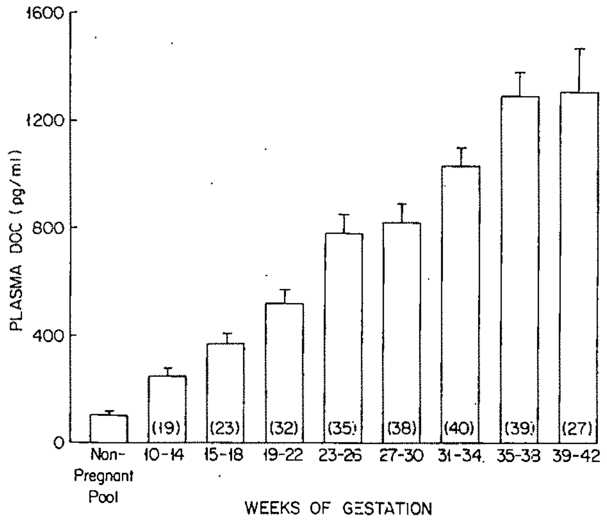

Fig. 1. Plasma concentrations of deoxycorticosterone *(DOC)* during uncomplicated pregnancies, as measured by radioimmunoassay in 44 women whose blood was sampled at approximately 2-week intervals. The data are shown for 4-week intervals and are presented as the mean ± standard error of the mean. The number of women for whom plasma DOC was determined in each sampling period is shown in parentheses in each vertical bar. The concentration of DOC in a pool of plasma derived from men and nonpregnant women is also depicted.

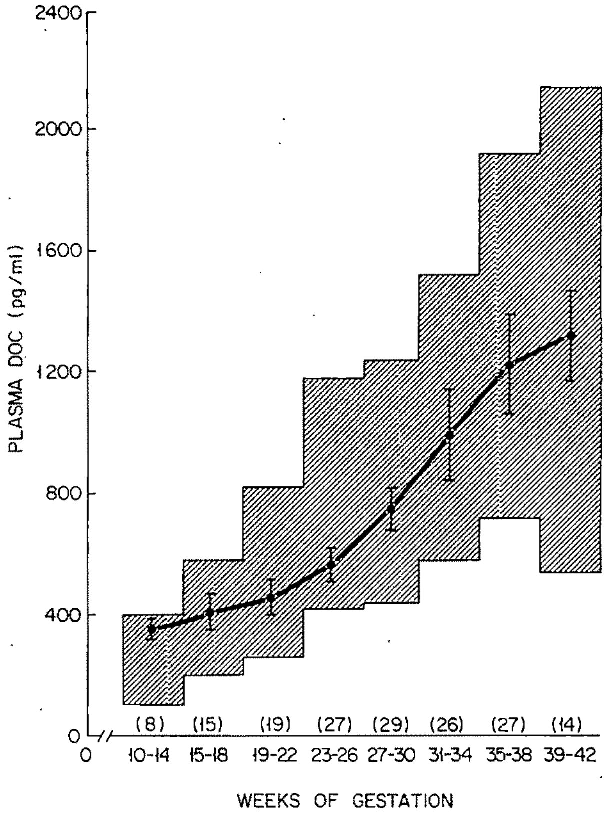

Fig. 2. Plasma concentrations of deoxycorticosterone *(DOC)* during gestation in 31 women who developed pregnancy-induced hypertension. Measurements were made at approximately 2-week intervals. The number of women in whom plasma DOC was determined in each study period is shown in parentheses. The data for this group are presented as the mean ± standard error of the mean. The hatched area represents the mean ± 1 standard deviation for DOC levels in the women whose pregnancies remained normal.

tetrahydro-DOC, and 17α-hydroxypregnenolone. The antiserum cross reacted slightly with 17,21-dihydroxy-4-pregnen-3,20-dione (3.3%) and 21- hydroxypregnenolone (3%). The only compound which cross reacted significantly with the antiserum, other than DOC (100%), was progesterone (50%), which was completely separated from DOC during column chromatographic isolation of DOC from the plasma extracts.

The radioimmunoassay of DOC was conducted as follows: The column fraction containing DOC was evaporated and reconstituted in 0.1M phosphate buffer, pH 7.0, containing 0.1% gelatin (assay buffer). DOC standards, in concentrations ranging from 3 to 400 pg/0.4 ml, were prepared similarly in assay buffer. Standards and unknowns (the equivalent of 25% of the plasma extract) were incubated in duplicate with antiserum (diluted 1:5,000 in assay buffer) and 5,000 cpm [^{3}H]DOC. After an overnight incubation at 4° C, 0.2 ml of dextran-coated charcoal suspension was added. The tubes were mixed and incubated for 10 minutes at 4° C, after which they were centrifuged at 2,000 rpm for 10 minutes at 4° C. The supernatant fluid (containing antibody-bound steroid) was decanted and mixed with liquid scintillation fluid, and radioactivity was assayed in a Packard TriCarb liquid scintillation spectrometer. The efficiency for tritium was 59%. The concentrations of DOC in unknown samples were computed through the use of a standard line and were corrected for procedural losses by the use of the internal recovery tracer. The sensitivity of the assay was 6 pg, and the least amount of DOC detectable in the assay method, assuming 100% recovery of DOC during extraction and chromatography, was 24 pg DOC per milliliter of plasma. To evaluate the accuracy of the method, known quantities of nonradiolabeled DOC (100, 200, 300, or 400 pg) were added in triplicate to 1.0-ml aliquots of a plasma pool, containing 103 pg DOC per milliliter, which were then assayed for DOC as described above. The differences in concentrations of DOC between the plasma pool with no DOC added and those samples to which DOC was added were 99, 197, 295, and 393 pg, respectively. Within-assay and between-assay variations for the DOC radioimmunoassay procedure were 7% and 17%, respectively. Plasma

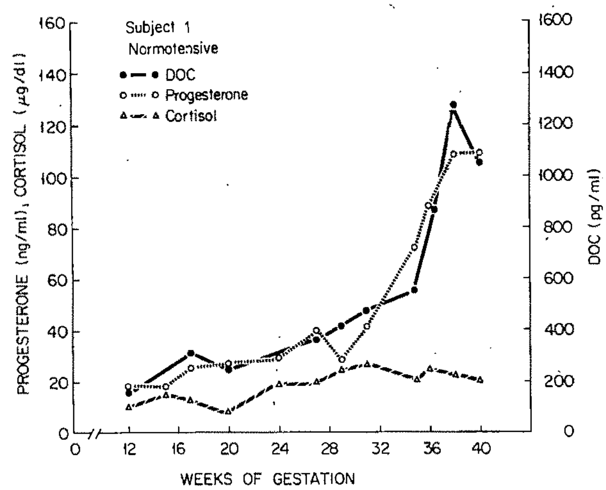

Fig. 3. Plasma levels of deoxycorticosterone *(DOC)*, progesterone, and cortisol throughout gestation in women whose pregnancy was normal.

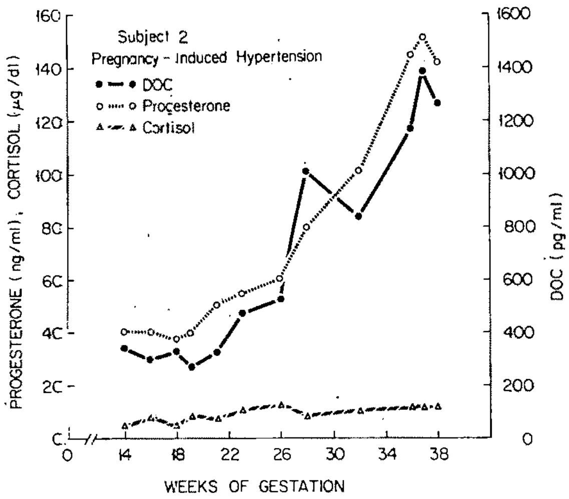

Fig. 4. Plasma levels of deoxycorticosterone *(DOC)*, progesterone, and cortisol throughout gestation in a woman who developed pregnancy-induced hypertension.

levels of progesterone and cortisol were also quantified in some selected samples by radioimmunoassay methods described in detail elsewhere.[8, 9] Each assay conducted in this study contained samples of plasma from subjects whose pregnancies remained normal and from those who developed PIH.

Results

Plasma levels of DOC during normal primigravid pregnancy. The plasma levels of DOC throughout the gestational period in 44 primigravid women who had uncomplicated pregnancies are illustrated in Fig. 1. In these women, the plasma concentrations of DOC increased progressively throughout gestation. Near the end of the first trimester (10 to 14 weeks), the plasma level of DOC was 234 ± 33 pg/ml (mean ± SE). By the end of the second trimester (23 to 26 weeks), the plasma level of DOC had risen to 778 ± 65 pg/ml. At term, the mean plasma concentrations of DOC ranged from 1,292 ± 96 pg/ml (35 to 38 weeks) to 1,309 ± 155 pg/ml (39 to 42 weeks), levels which are about twelve times that in pooled plasma from men and nonpregnant women (112 ± 20 pg/ml, mean ± SD).

Plasma levels of DOC in primigravid women who developed PIH. The plasma concentrations of DOC throughout the gestational period in 31 primigravid women who developed PIH during the third trimester are illustrated in Fig. 2. As in the women whose pregnancies remained normal, the plasma levels of DOC in women destined to develop PIH increased progressively throughout the gestational period. Near the end of the first trimester (10 to 14 weeks), the plasma level of DOC in these women was 346 ± 34 pg/ml. By the end of the second trimester, the plasma level of DOC had risen to 547 ± 56 pg/ml. Near term, at which time most of these women developed PIH, the plasma concentrations of DOC ranged from 1,206 ± 166 pg/ml (35 to 38 weeks) to 1,297 ± 150 pg/ml (39 to 42 weeks). At no time during their pregnancy were the plasma concentrations of DOC in these women, as a group, significantly different from those of the primigravid women who remained normotensive (Fig. 2). Moreover, there was no apparent relationship between the eventual severity of PIH and the plasma levels of DOC during gestation.

Relationship of plasma levels of DOC to plasma levels of progesterone and cortisol. DOC, progesterone, and cortisol in plasma were measured throughout gestation in one woman with a normal pregnancy and in one woman whose pregnancy was complicated by PIH. In both women, the plasma levels of DOC fluctuated in a manner remarkably similar to those of progesterone but not of cortisol (Figs. 3 and 4). Indeed, plasma levels of DOC and progesterone increased fivefold to sixfold throughout gestation in the woman who had an uncomplicated pregnancy (Fig. 3) and in the woman who developed PIH (Fig. 4). However, in both women, cortisol levels increased only twofold during pregnancy. In other subjects studied (data not shown), a similar parallelism between DOC and progesterone was observed, but neither progesterone nor DOC values corresponded to changes in plasma cortisol levels. This finding suggests that the source of the increased plasma concentrations of DOC during pregnancy may be peripheral conversion of circulating progesterone to DOC.

Comment

The physiologic adaptations of pregnant women to the ever-changing hormonal milieu of pregnancy are remarkable. Perhaps the most striking adaptation is the capacity to maintain normal salt and water balance as well as blood pressure at a time when the production of mineralocorticosteroids, such as aldosterone and deoxycorticosterone, is elevated greatly over that in the nonpregnant state.[1, 2, 4, 5, 10-14, 17] Unfortunately, some women, especially primigravidas, for reasons not yet well understood, develop salt and water retention, edema, and hypertension, i.e., pregnancy-induced hypertension. Although there is no convincing evidence at present to support the viewpoint that an excessive production or manifestation of mineralocorticosteroid(s) could be involved in the pathogenesis of PIH,[3] prior studies were performed only after the women had developed PIH.[1, 4, 14]

In the current investigation, measurements were made throughout gestation in a large group of primigravid women in order to ascertain whether any derangement in the plasma levels of DOC could be detected either before or after the development of PIH. The results obtained in the current study are in agreement with those of Brown and associates[1] and Nolten and associates,[2, 5] i.e, plasma concentrations of DOC in pregnant women are increased over those in nonpregnant subjects. Values of plasma DOC were elevated as early as 10 to 14 weeks' gestation, the earliest time during pregnancy studied; thereafter, they rose progressively throughout pregnancy, reaching about 1,300 pg/ml during the last 8 weeks of gestation in women whose pregnancies remained normal. In agreement with Brown and associates,[1] we found that the plasma levels of DOC in women who had developed PIH were similar to those in normotensive women during the third trimester. Brown and associates' findings[1] are in contrast to those of Weir and associates,[4] who reported that plasma levels of aldosterone and DOC in women with hypertension and proteinuria were one half the concentrations measured in normal pregnant women. Since none of our six patients who developed severe hypertension was studied after the onset of symptoms, we are unable to corroborate or refute that finding of Weir and associates.[4] The plasma levels of DOC in both the normal and hypertensive subjects in the study of Weir and associates[4] were substantially lower than those found in the present study and reported by others.[1, 2, 5, 11] The reason for this discrepancy is unclear. In contrast to the experimental design of the studies previously cited[1, 4, 14] that dealt with mineralocorticosteroids and PIH, we were able to evaluate a large number of women prospectively. Women who ultimately developed PIH had plasma levels of DOC that were similar to those in women who remained normotensive Therefore, we conclude that the development of PIH in these primigravidas was not singularly the result of excessive circulating levels of DOC.

Such a conclusion does not preclude the possibility that tissue levels of DOC, in the kidney, for instance, might not be excessive in such women. This possibility deserves some consideration for several reasons. It is clear from the results of several studies[1, 2, 5, 11] that, at least in the latter stages of pregnancy, the production of DOC is *not* influenced by ACTH in a manner similar to that in nonpregnant individuals.[12] This obtains since (a) DOC levels do not decline after the administration of dexamethasone, whereas cortisol levels are suppressed,[1, 2, 5] and (b) although the levels of DOC are responsive to stimulation by ACTH in nonpregnant subjects and in women during the first and second trimesters, they are only marginally stimulated by ACTH during the third trimester.[2, 5, 14] In addition, we and others[2] have found that the temporal patterns of DOC and cortisol are divergent throughout the course of gestation. In this regard, we found that the levels of DOC in normotensive women, as well as in those women destined to develop PIH, fluctuated in a manner which was remarkably similar to that of progesterone. In view of the fact that plasma levels of DOC corresponded well to those of progesterone throughout gestation, and since we found previously that circulating progesterone could be metabolized to DOC in pregnant women as well as in nonpregnant subjects,[6, 7] progesterone may be an important source of DOC in pregnancy, especially during the third trimester, when circulating levels of progesterone range from 100 to 200 ng/ml in primigravid subjects.[15] If this is the case, then the somewhat confusing reports concerning dexamethasone suppression and ACTH stimulation of DOC during pregnancy may be explained more easily. This explanation of the origin of DOC is an alternative to the proposal that maternal DOC may arise from the fetus.[2] The fetus may, indeed, synthesize substantial amounts of DOC, but on the basis of blood flow from the fetus to the placenta and the arteriovenous difference in umbilical cord plasma levels of DOC,[1] the fetus cannot be a significant source of the DOC in the maternal circulation. At present, the information concerning the clearance of DOC-sulfate in the fetus by the placenta is insufficient for evaluation of this potential source of entry of DOC into the maternal compartment.

If much of the DOC in plasma during the third trimester were to arise via 21-hydroxylation of circulating progesterone in the maternal compartment, and if some of this conversion were to take place in the maternal kidney,[16] then excessive local tissue concentrations of DOC could occur in some women in whom this enzyme activity is great. If this were the case, no significant elevation in plasma levels of DOC would occur, although the effective physiologic concentrations in a receptive tissue site might be excessive. Thus, as suggested several years ago by Rabb and associates,[3] PIH may be related to increased production of mineralocorticosteroid(s). However, the biosynthesis of such a steroid, e.g., DOC, in excessive amounts might be masked if the site of production were the kidney, where synthesis from progesterone and biologic action could occur within the single organ.

We acknowledge the able technical assistance of Loretta Dice, M. Louise Hall, Shelton Ragland, George Crowley, and Julia Morrison, and the excellent editorial assistance of Millie Arnold.

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Prolactin modulation of dehydroepiandrosterone sulfate secretion

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To clarify the controversy about the effect of prolactin (PRL) on dehydroepiandrosterone sulfate (DHEA-S), this study was undertaken to investigate the effects of alterations in plasma PRL on plasma DHEA-S concentrations in hyperprolactinemic women, as well as in normal male subjects. DHEA-S was measured in a group of 21 women with hyperprolactinemia, galactorrhea, and amenorrhea (PRL: 257 ± 89 ng/ml; mean ± SEM). In these women, mean plasma concentrations of DHEA-S (2.54 ± 0.2 μg/ml) were significantly higher ($p < 0.005$) than those in 41 normal control women (1.78 ± 0.1 μg/ml) and those in a group of 11 amenorrheic patients (1.77 ± 0.2 μg/ml). Eight women with hyperprolactinemia were given 5 mg of bromocriptine each day for 4 consecutive weeks. Within 1 week of medication, PRL levels fell by 60% ($p < 0.05$). To test whether lowering normal plasma levels of PRL would affect plasma concentrations of DHEA-S, five normal male subjects received a 48-hour infusion of dopamine at an average rate of 6 μg/kg/min. Plasma levels of PRL fell by 60% ($p < 0.01$) after 8 hours of infusion, and DHEA-S decreased by 27% by 16 hours ($p < 0.05$). These data suggest that PRL modulates the secretion of DHEA-S: an increase in plasma levels of PRL is correlated with elevated concentrations of DHEA-S, whereas a decrease in PRL is followed by a fall in DHEA-S. (AM. J. OBSTET. GYNECOL. 138:632, 1980.)

SEVERAL investigators have presented data linking serum concentrations of prolactin (PRL) to adrenal production of C-19 steroids. This association is plausible since there are known PRL receptor sites in the adrenal glands.[1-3]

Dehydroepiandrosterone sulfate (DHEA-S) is quantitatively the most important C-19 steroid produced by the adrenal glands. Elevated levels of plasma DHEA-S have been found in women with hyperprolactinemia and amenorrhea, but not in women with amenorrhea alone.[4] Giving sulpiride, 100 mg daily, to elevate PRL, Vermeulen and associates[5] observed increases in DHEA-S in two normal women. Similar results were obtained in postmenopausal women who were taking psychotropic drugs. Carter and associates[6] showed elevations in DHEA-S in women with amenorrhea and hyperprolactinemia which could be lowered by administering bromocriptine. Maroulis and Sherman[7] reported decreases in DHEA-S after operations for PRL-secreting pituitary tumors. Nevertheless, there is evidence to the contrary, since other authors were unable to find an association between PRL and DHEA-S. Parker and associates[8] and Metcalf and associates[9] could not show elevations in hyperprolactinemic patients. Wiebe and Handwerger[10] could not alter serum DHEA-S levels by administering chlorpromazine for 4 hours in order to induce hyperprolactinemia.

The elevations of DHEA-S in patients with hyperprolactinemia are not thought to be the result of measured levels of adrenocorticotropic hormone (ACTH) in the blood. Caufriez and associates[11] have found that

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This work was supported in part by Grant HD-05932 and by General Clinical Research Grant RR-43 from the National Institutes of Health, United States Public Health Service, Bethesda, Maryland.

Sponsored by the Society for Gynecologic Investigation.

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**Recipient of a Medical Research Council of Canada Fellowship, 1977 to 1980.*

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patients with hyperprolactinemia have normal or decreased levels of ACTH and normal levels of cortisol.

The present study was undertaken to attempt clarification of the relationship between PRL and DHEA-S. The aim of this study was to investigate: (1) whether elevations and decreases in PRL correlate with changes in serum DHEA-S, and (2) whether an acute decrease in PRL levels would be followed by falling serum concentrations of DHEA-S while secretion of ACTH would remain unchanged as suggested by unaltered plasma levels of cortisol.

Material and methods

Eighty-eight individuals participated in this study which had been approved by the institutional research committee after informed consent was obtained from each volunteer. The following groups of volunteers were investigated. Forty-one normal women of reproductive age who had regular menstrual cycles and normal plasma levels of PRL were included as control subjects in this study. Antecubital venous blood was drawn during the luteal phase between 8:00 A.M. and 12:00 noon. Eleven women with amenorrhea but without galactorrhea, and 21 women with amenorrhea and galactorrhea were included in this study. The former group of 11 women with amenorrhea had normal serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and PRL and responded with uterine bleeding to the intramuscular administration of progesterone, thus indicating normal estrogen status. This group of women, therefore, were considered to have hypothalamic dysfunction.

The group of 21 women with amenorrhea-galactorrhea and hyperprolactinemia had normal or low serum levels of LH and FSH. Only three of these women had uterine bleeding after intramuscular progesterone. These three women and 11 others had normal polytomograms of the sella turcica (idiopathic hyperprolactinemia), whereas the other seven had abnormal x-ray films compatible with pituitary adenoma. All seven of these women had a normal response of cortisol to insulin-induced hypoglycemia.

In order to investigate whether a lowering of elevated plasma PRL would reduce circulating DHEA-S, eight additional young females in the reproductive age group with amenorrhea and hyperprolactinemia were treated with 5 mg of bromocriptine (2-bromo-α-ergocriptine mesylate) daily for 4 weeks. Samples of blood were obtained once a week during these 4 weeks.

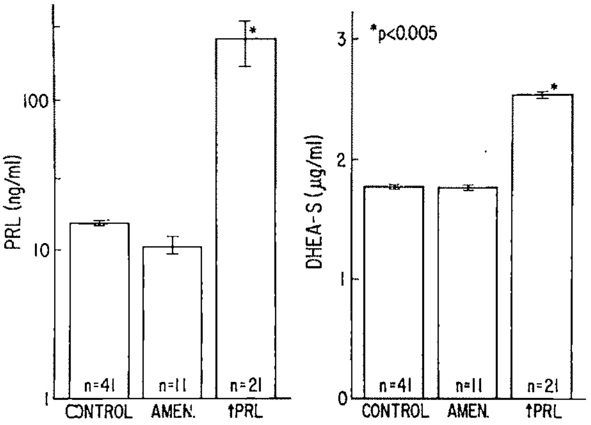

Fig. 1. Mean ± SEM of serum concentrations of PRL and DHEA-S in normal, amenorrheic, and hyperprolactinemic women.

To study whether a diminution in normal plasma concentration of PRL would result in a reduction in circulating DHEA-S, five healthy men with normal plasma concentrations of PRL were given a 48-hour intravenous infusion of dopamine. The men were admitted to the Clinical Research Center and kept under resting conditions for 24 hours both before and after the infusion. The minimal dose of dopamine required to raise either the systolic blood pressure by 10 mm Hg or the heart rate by 15 bpm was administered to a given volunteer. The doses of dopamine given averaged 6 μg/kg/min. Within 2 hours after completion of the infusion, the blood pressures and heart rates returned to normal. Venous blood was collected at 8-hour intervals.

Plasma was stored at −20° C until it was analyzed for PRL,[13] DHEA-S,[14] and cortisol[15] by the radioimmunoassay (RIA) procedures previously described. Cortisol was assayed only in samples obtained after infusion of dopamine. A rabbit antidehydroepiandrosterone-3-hemisuccinyl-bovine serum albumin serum raised in our laboratory was similar to the antiserum described by Buster and Abraham.[14] Our assay for DHEA-S gave results comparable to those found in the literature.[4-6, 11] In addition, measurement of DHEA-S by a procedure involving solvolysis, chromatography on Sephadex LH-20 columns, and gas-liquid chromatography/mass spectrometry in serum aliquots also subjected to direct RIA indicated that the DHEA-S fraction measured by our RIA was indeed DHEA-S. Androstenedione, androst-5-ene-3,17-dione, and DHEA were found to cross react 21.2%, 65.5%, and 100%, respectively, with DHEA-S, whereas etiocholanolone and androsterone cross reacted less than 0.1%. Because of the 1,000-fold concentration difference between these unconjugated androgens present in nanograms per milliliter concentrations and DHEA-S at micrograms per milliliter levels, steroids such as DHEA account for only 0.1% or less of the DHEA-S measured. Interassay and intra-assay co-

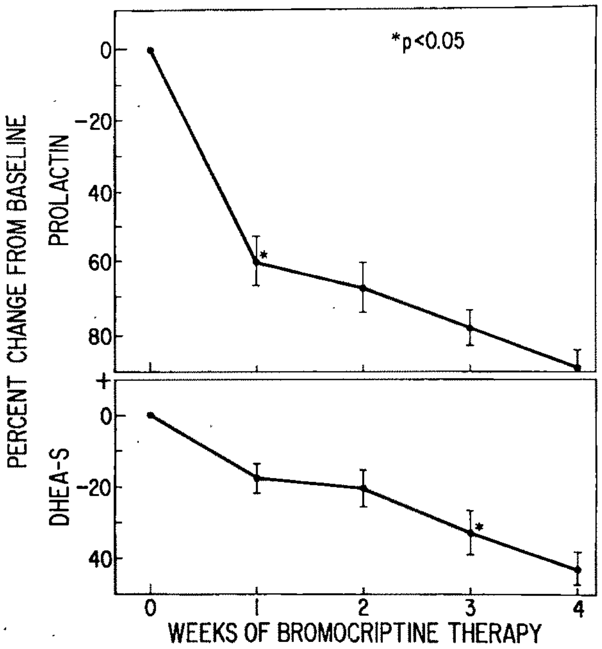

Fig. 2. Mean percentage changes (±SEM) in serum concentrations of PRL and DHEA-S in eight hyperprolactinemic women in response to a daily dose of 5 mg of bromocriptine.

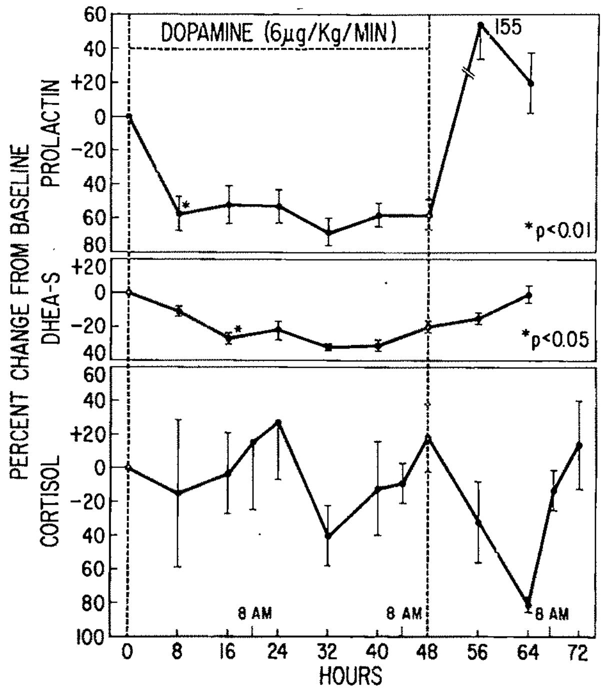

Fig. 3. Mean percantage changes (±SEM) in serum concentrations of PRL, DHEA-S, and cortisol in five normal men during and after intravenous infusion of dopamine.

efficients of variation for DHEA-S averaged 6.5% and 10%, respectively. Student's t test was used for statistical analysis.

Results

The mean (± SEM) plasma concentrations of DHEA-S found in the 41 control subjects (1.78 ± 0.1 μg/ml) and in the 11 amenorrheic patients (1.77 ± 0.2 μg/ml) did not differ from each other. The mean plasma levels of DHEA-S (2.54 ± 0.2 μg/ml) and PRL (257 ± 89 ng/ml) measured in the 21 amenorrheic and hyperprolactinemic women were significantly higher ($p < 0.005$) than both the DHEA-S and PRL concentrations in the normal control women and the amenorrheic women without galactorrhea (Fig. 1).

Plasma levels of PRL fell, on the average, by 60% ($p < 0.005$) in response to bromocriptine within 1 week after initiation of therapy, whereas 3 weeks of bromocriptine therapy was required before a 32% ($p < 0.05$) fall in plasma DHEA-S was achieved (Fig. 2).

Plasma PRL levels fell by 60% ($p < 0.01$) from baseline levels within 8 hours after the start of the dopamine infusion whereas plasma DHEA-S concentrations had decreased at 16 hours by only 27% ($p < 0.05$). At the end of the infusion of dopamine, plasma levels of PRL exhibited a rebound rise and achieved a mean increase to 155% of preinfusion baseline values. Plasma levels of DHEA-S did exhibit a rebound surge, but returned slowly to baseline values. Plasma levels of cortisol did not vary significantly either during or after infusion of dopamine and exhibited the usual diurnal variations (Fig. 3).

Comment

DHEA-S is quantitatively the most important adrenal C-19 steroid and is produced predominantly by the adrenal glands. Its metabolic clearance rate, estimated as 8 to 16 L/day,[16, 17] is very low and is due, in part, to its binding to serum albumin. Circulating DHEA-S represents a relatively large pool of at least 3.5 mg[18] and is not subject to diurnal fluctuations.[18, 19] Because DHEA-S has a long half life[20, 21] and is present as a large pool with a slow turnover, the adrenal glands must maintain increased secretion of it over several hours before the levels of it in peripheral plasma rise significantly. Nieschlag and associates[18] found that, in six of eight men, plasma levels of DHEA-S increased 1.3 to 2.5-fold over basal levels at the end of an 8-hour infusion of adrenocorticotropic hormone. For these reasons, it is difficult to elicit rapid changes in plasma levels of DHEA-S by stimulation or suppression of the adrenal glands.

The foregoing data which reveal increased plasma concentrations of DHEA-S in correlation with hyperprolactinemia disagree with the findings of some authors[8, 9] but are in agreement with the findings of most[4-7] whose reports state that hyperprolactinemia correlates with increased plasma DHEA-S. This study also confirms that patients with normoprolactinemic amenorrhea do not have elevated plasma levels of DHEA-S as reported by Bassi and associates.[4] Moreover, this study has shown that a lowering and raising of plasma PRL levels correlate with a decrease and an increase, respectively, in plasma concentrations of DHEA-S. Our data document that not only a lowering of elevated plasma PRL levels but also a diminution in normal plasma concentrations of PRL correlate with a decrease in circulating DHEA-S. Although these data strongly suggest an effect of PRL on the secretion of DHEA-S, definitive proof of association is lacking. The theoretical possibility exists that bromocriptine or dopamine may directly affect the secretion of DHEA-S in the patients studied.

Carter and associates[6] administered ACTH as a 4-hour intravenous infusion to hyperprolactinemic women and repeated this after PRL levels were normalized. These investigators found that ACTH-stimulated DHEA-S levels were not significantly different with high or with normal levels of PRL. Nevertheless, Kandeel and associates[22] administered ACTH once daily over a 4-day period and showed a greater incremental increase in DHEA-S in hyperprolactinemic patients than in normal control subjects.

As observed in our study with dopamine, levels of PRL had to be lowered for at least 16 hours before the first significant decline in the plasma concentration of DHEA-S was achieved. This was probably due to the slow turnover of circulating DHEA-S. The long half-life of DHEA-S may explain why some investigators[10] were not able to show an increase in plasma levels of DHEA-S after inducing hyperprolactinemia for only 4 hours. The fact that several hours of infusion of ACTH are required before plasma levels of DHEA-S rise significantly explains the discrepancy between the data of Carter and associates[6] and Kandeel and associates.[22] Being unable to obtain and use a drug which achieves a sustained hyperprolactinemia of 16 to 24 hours' duration, we were unable to examine whether the raising of normal PRL levels would result in increased plasma concentrations of DHEA-S.

The finding that the adrenal glands contain PRL receptors[1-3] supports the hypothesis that PRL has a modulating effect on DHEA-S. The mechanism by which PRL affects the secretion of DHEA-S is unknown. In the study of Kandeel and associates,[22] hyperprolactinemic women treated with ACTH had greater increases in circulating DHEA-S than in Δ^4-steroids, such as testosterone and cortisol. Those investigators hypothesized that this might indicate that in hyperprolactinemia there is a disturbance in the 3 β-ol-dehydrogenase enzyme system that favors the Δ^5-steroids. Other Δ^5-steroids have not been measured as yet, and more work is needed to elucidate this mechanism. Perhaps PRL plays a functional role in modulating the secretion of DHEA-S by affecting the 3 β-ol-dehydrogenase enzyme. Our observation in this study that a 60% reduction in serum PRL achieved an approximate 30% decrease in serum DHEA-S is consistent with a modulating role.

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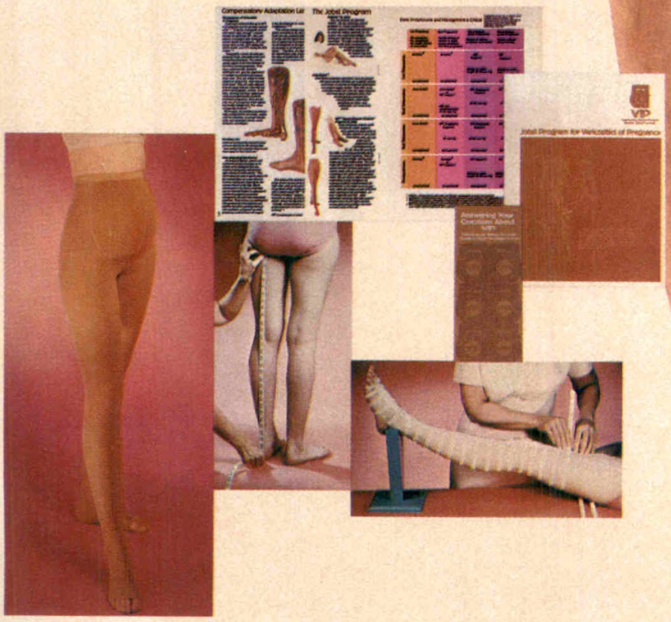
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RhoGAM

Rh$_o$(D) Immune Globulin (Human)

Indications: RhoGAM Rh$_o$ (D) Immune Globulin (Human) is used to prevent formation of active anti-Rh$_o$ (D) antibody in an Rh$_o$ (D) negative, D^u negative individual into whose bloodstream Rh$_o$ (D) positive or D^u positive blood has entered. Such an occurrence might result from full term or early termination of an Rh incompatible pregnancy, or from an Rh incompatible transfusion.

Dosage: One vial of RhoGAM will completely suppress immunity to 15 ml of Rh positive red blood cells (packed cells, not whole blood). In deciding the dose of RhoGAM which is to be administered to an individual, the volume of Rh positive blood which entered the person's bloodstream is to be determined, from which the dose of RhoGAM can be calculated. In the usual full-term delivery, a single dose (one vial) of RhoGAM will be adequate to suppress immunity to the Rh$_o$ (D) antigen. However, in the case of a large feto-maternal hemorrhage or an Rh incompatible transfusion accident, more than one dose may be indicated.

Product Description: RhoGAM is a sterile concentrated solution of specific immunoglobulin (IgG) containing anti-Rh$_o$ (D) fractionated from carefully screened human plasma by a cold alcohol method.

Action: With an injection of passive Rh$_o$ (D) antibody (RhoGAM) to the postpartum mother, a postmiscarriage/abortion woman, or to the recipient of a transfusion accident, the person's antibody response to the foreign Rh$_o$ (D) positive cells is suppressed.

Cautions: Reactions of Rh$_o$ (D) negative individuals given Rh$_o$ (D) Immune Globulin (Human) are infrequent, of a mild nature and mostly confined to the area of injection. An occasional patient may react more strongly, both locally and generally. A slight elevation of temperature has been noted in a small number of postpartum women.

Fever, myalgia, lethargy, increased bilirubin levels and/or splenomegaly have been infrequently observed in individuals following a multi-dose regimen after Rh mismatched transfusions.

Systemic reactions are rare and sensitization due to repeated injections of immune globulins is unusual.

Immune Serum Globulin (Human) has not been reported to transmit hepatitis.

RhoGAM is to be given to the recipient in a transfusion accident or the postpartum mother or postmiscarriage woman, only. It must not be given to the infant.

Contraindications: Rh$_o$ (D) Immune Globulin (Human) should *not* be administered to:

1. An Rh$_o$ (D) positive or D^u positive individual.
2. An Rh$_o$ (D) negative patient who has inadvertently received an Rh$_o$ (D) positive blood transfusion within three months of delivery.
3. A patient previously immunized to the Rh$_o$ (D) blood factor.

Supplied: In vials containing a single dose of RhoGAM. Store at 2° to 8° C. Do not freeze.

NOTE: *For complete prescribing information, see direction circular.*

Twenty thousand infants owe their lives to RhoGAM*

$Rh_o(D)$ Immune Globulin (Human)

...and to you.

Since RhoGAM was first administered, over a decade ago, an estimated one hundred thousand sensitizations have been avoided, and twenty thousand fetal deaths from Rh hemolytic disease have been prevented.[1]

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We have acted on our commitment with products and services to spread knowledge and understanding of the need to protect. We have sponsored continuing research and education with international symposia, books, pamphlets and films. We have helped design a record-keeping system to improve utilization, and have provided support to the medical profession with the RhoGAM Protection Policy.

We are doing all we can to help you help every patient at risk. There is still more to be done, and together we will find the way!

RhoGAM... the proven product.

[1] Center for Disease Control: Rh Hemolytic Disease: Surveillance, August 1976, Revision, DHEW Publication No. (CDC) 75-8310, Atlanta, 1976.

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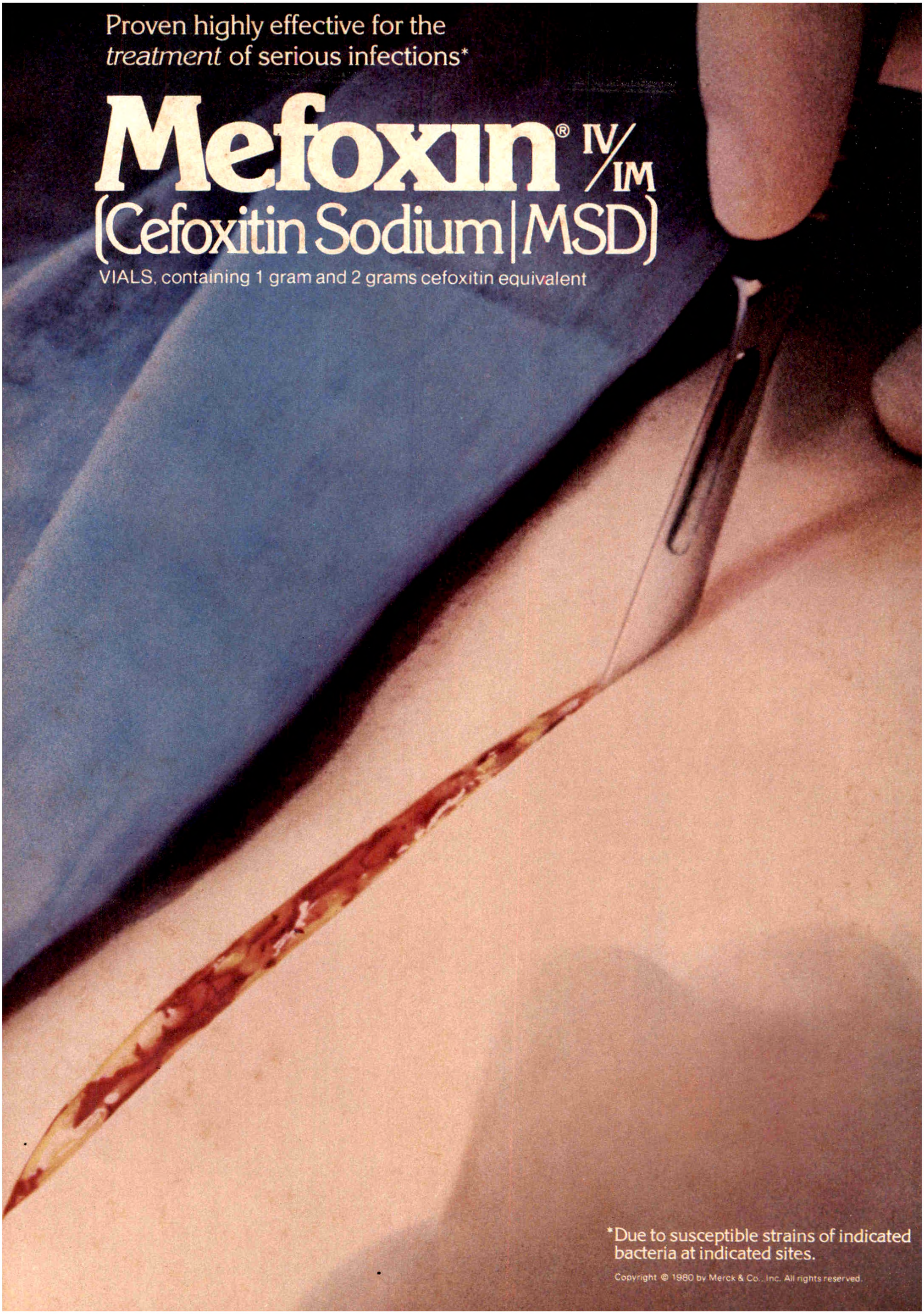

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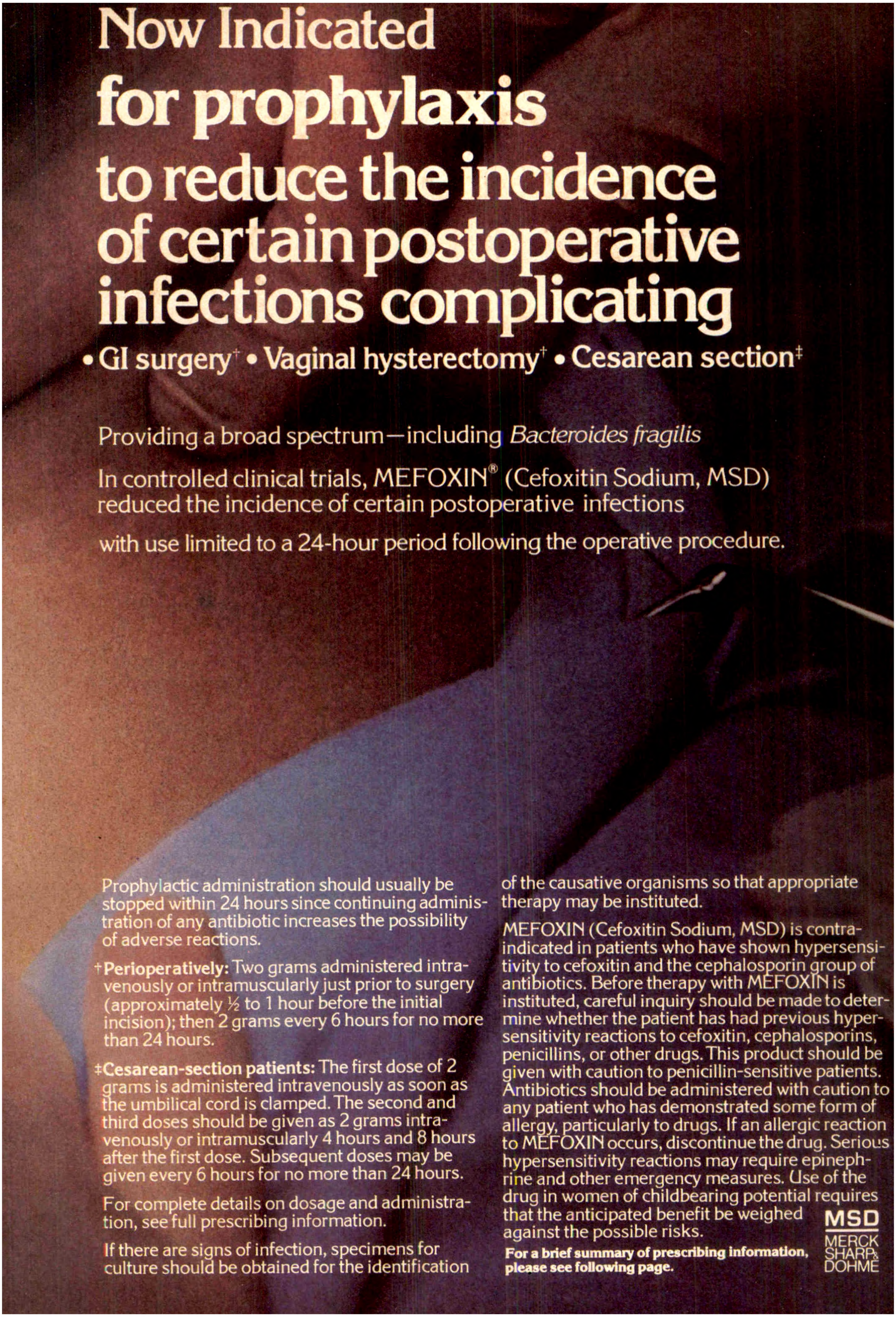

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with use limited to a 24-hour period following the operative procedure.

Prophylactic administration should usually be stopped within 24 hours since continuing administration of any antibiotic increases the possibility of adverse reactions.

[†] **Perioperatively:** Two grams administered intravenously or intramuscularly just prior to surgery (approximately ½ to 1 hour before the initial incision); then 2 grams every 6 hours for no more than 24 hours.

[‡] **Cesarean-section patients:** The first dose of 2 grams is administered intravenously as soon as the umbilical cord is clamped. The second and third doses should be given as 2 grams intravenously or intramuscularly 4 hours and 8 hours after the first dose. Subsequent doses may be given every 6 hours for no more than 24 hours.

For complete details on dosage and administration, see full prescribing information.

If there are signs of infection, specimens for culture should be obtained for the identification of the causative organisms so that appropriate therapy may be instituted.

MEFOXIN (Cefoxitin Sodium, MSD) is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics. Before therapy with MEFOXIN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefoxitin, cephalosporins, penicillins, or other drugs. This product should be given with caution to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to MEFOXIN occurs, discontinue the drug. Serious hypersensitivity reactions may require epinephrine and other emergency measures. Use of the drug in women of childbearing potential requires that the anticipated benefit be weighed against the possible risks.

For a brief summary of prescribing information, please see following page.

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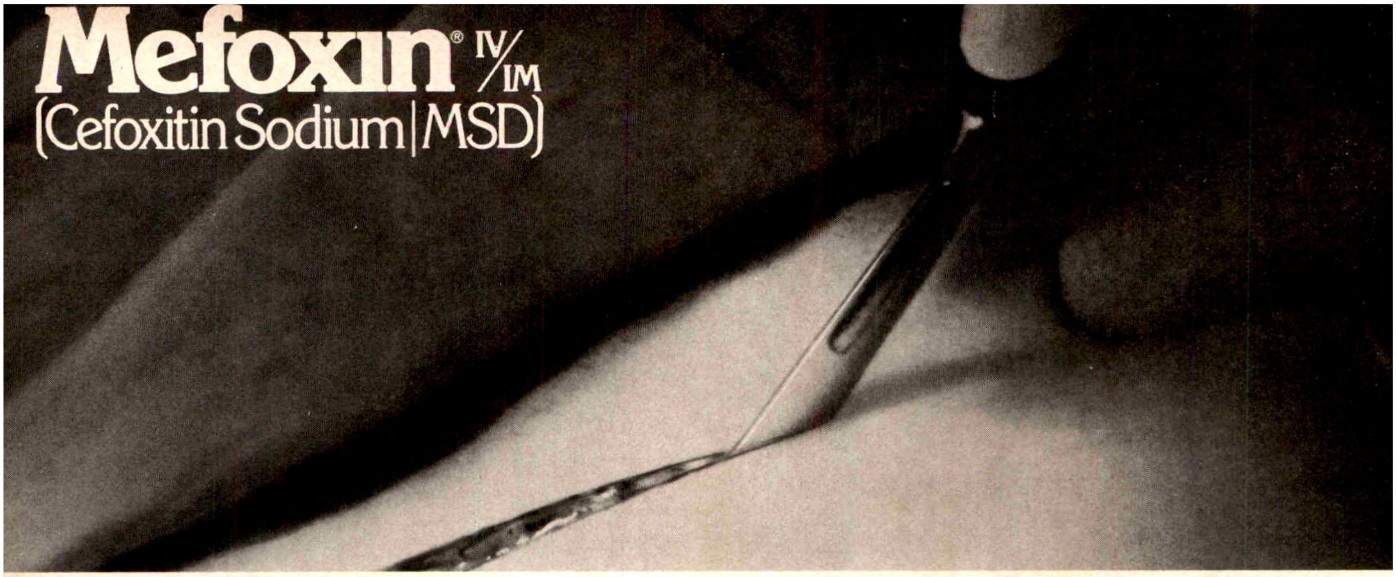

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Indications and Usage: ***Treatment***– Serious infections caused by susceptible strains of the designated microorganisms in the following diseases:
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GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli, Klebsiella* species, *Proteus mirabilis,* indole-positive *Proteus* (i.e., *P. morganii, P. rettgeri,* and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae.*
INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli, Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,‡ and *Clostridium* species.
GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli, N. gonorrhoeae, Bacteroides* species including the *B. fragilis* group,‡ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.
SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli, Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.‡
BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).
SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis,* streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli, P. mirabilis, Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,‡ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.
Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae.* Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.
Prevention– Prophylactic use perioperatively (preoperatively, intraoperatively, and postoperatively) in surgical procedures (e.g., vaginal hysterectomy, gastrointestinal surgery) classified as contaminated or potentially contaminated or in patients in whom infection at the operative site would present a serious risk, e.g., prosthetic arthroplasty; intraoperatively (after umbilical cord is clamped) and postoperatively in cesarean section.
MEFOXIN usually should be given ½ to 1 hour before the operation, which is sufficient time to achieve effective levels in the wound during the procedure. Prophylactic administration should usually be stopped within 24 hours since continuing administration of any antibiotic increases the possibility of adverse reactions but, in the majority of surgical procedures, does not reduce the incidence of subsequent infection. However, in patients undergoing prosthetic arthroplasty, it is recommended that MEFOXIN be continued for 72 hours after the surgical procedure. If there are signs of infection, specimens for culture should be obtained for the identification of the causative organism so that appropriate therapy may be instituted.
Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.
Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.
Precautions: The total daily dose should be reduced in patients with transient or persistent reduction of urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur in such individuals from usual doses. As with other antibiotics, prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures. Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.
Interference with Laboratory Tests—As with cephalothin, high concentrations (>100 mcg/ml) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. A false-positive reaction for glucose in urine has been observed with CLINITEST§ reagent tablets.
Pregnancy—In women of childbearing potential, weigh anticipated benefit against possible risks.
Nursing Mothers—Cefoxitin is excreted in human milk in low concentrations.
Infants and Children—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.
Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash, pruritus, eosinophilia, fever, and other allergic reactions. *Gastrointestinal*—Nausea, vomiting, and diarrhea. *Blood*—Transient eosinophilia, leukopenia, neutropenia, and hemolytic anemia; a positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels.
Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.
How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent.

For more detailed information, consult your MSD representative or see full prescribing information. Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, PA 19486

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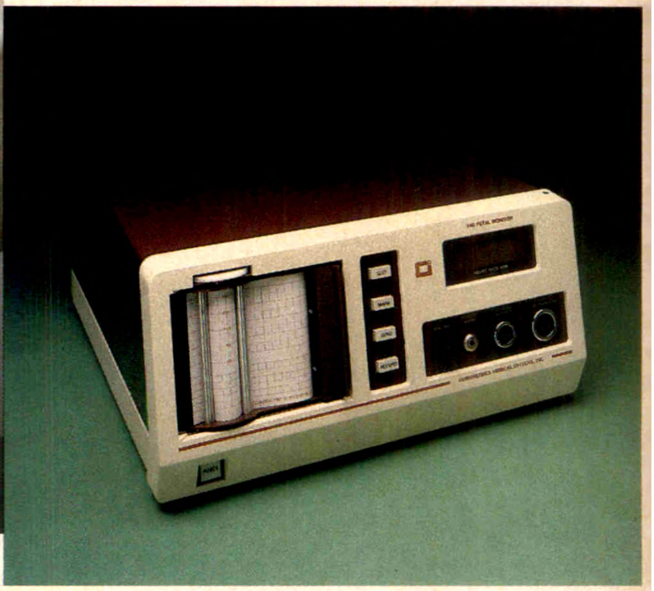

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Schifrin BS, Foye G, Amato J, Kates R, MacKenna J: outine Fetal Heart Rate Monitoring in the Antepartum eriod. Obstet Gynecol, 54: 21-25.
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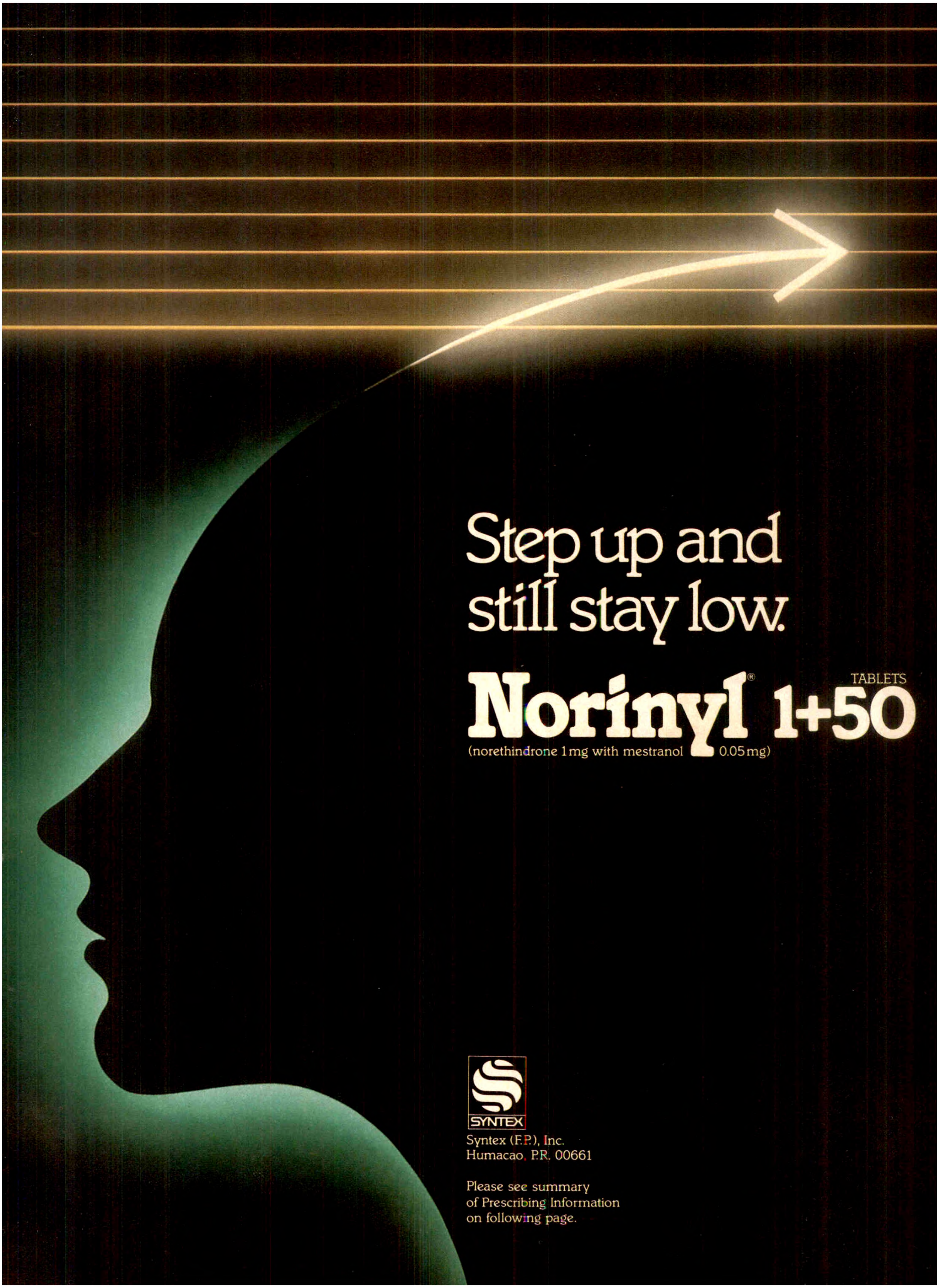

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ORAL CONTRACEPTIVE (O.C.) AGENTS

INDICATIONS O.C.s are indicated for the prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM FROM O.C.s.* Studies have shown a positive association between the dose of estrogens in O.C.s and the risk of thromboembolism. For this reason, it is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The O.C. product prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance. It is recommended that new acceptors of O.C.s should be started on preparations containing 0.05 mg or less of estrogen. **CONTRAINDICATIONS** 1. Known or suspected pregnancy (see Warning No. 5). 2. Thrombophlebitis or thromboembolic disorders. 3. A past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. O.C.s should not be used by women who have or have had any of the following conditions: a. Cerebral vascular or coronary artery disease. b. Known or suspected carcinoma of the breast. c. Known or suspected estrogen dependent neoplasia. d. Benign or malignant liver tumor which developed during the use of oral contraceptives or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from O.C. use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use O.C.s should be strongly advised not to smoke.

The use of O.C.s is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing O.C.s should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of O.C.s is well established. One British study demonstrated an increased relative risk for fatal venous thromboembolism; two British and three American studies demonstrated an increased relative risk for non-fatal venous thromboembolism. These studies estimate that users of O.C.s are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke, which had not been shown in prior British studies. In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the relative risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between O.C. use and stroke. Another British long-term, follow-up study had suggested such an association in 1974, but the number of cases was too small to estimate the risk. MYOCARDIAL INFARCTION. An increased relative risk of myocardial infarction associated with the use of O.C.s has been reported, confirming a previously suspected association. One study conducted in the United Kingdom found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an O.C. user or not. O.C.s, however, were found to be an additional risk factor. In terms of relative risk, it has been estimated that O.C. users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. O.C. users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, and about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the number of cigarettes smoked is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. (The estimates are based on British vital statistics which show acute myocardial infarction death rates 2- to 3-times less than in the U.S. for women in these age groups; consequently, actual U.S. death rates could be higher.) The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in O.C.s. Preparations containing 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been supported by a study in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only O.C.s have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASE. A large British prospective study estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of O.C.s according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for O.C. users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). The risk is concentrated in older women, in those with a long duration of use, and in cigarette smokers, and may persist after discontinuation of O.C. use. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of O.C.s) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The findings of this analysis are shown in Table 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that of childbirth, with the exception of O.C.s in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates given would be about double; for "light" smokers (less than 15 cigarettes a day), about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The study also concluded that O.C. users who smoke, especially those over the age of 30, have a greater mortality risk than O.C. users who do not smoke.

TABLE 1. *The annual number of deaths associated with control of fertility and no control per 100,000 nonsterile women by regimen of control and age of women are:*

•	15-19	20-24	25-29	30-34	35-39	40-44
No method	5.6	6.1	7.4	13.9	20.8	22.6
Abortion only	1.2	1.6	1.8	1.7	1.9	1.2
Pill only —nonsmokers	1.3	1.4	1.4	2.2	4.5	7.1
Pill only —smokers	1.5	1.6	1.6	10.8	13.4	58.9
IUDs only	0.9	1.0	1.2	1.4	2.0	1.9
Traditional contraception only	1.1	1.6	2.0	3.6	5.0	4.2
Traditional contraception and abortion	0.2	0.2	0.3	0.3	0.3	0.2

The risk of thromboembolic and thrombotic disease associated with O.C.s increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclampic toxemia and especially by cigarette smoking. Based on

sociated with use of O.C.s

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (No predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A—Use associated with very high risk. C—Use associated with moderate risk.
B—Use associated with high risk. D—Use associated with low risk.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of post-surgery thromboembolic complications has been reported in O.C. users. If feasible, O.C.s should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. Data also suggest that the presence of varicose veins substantially increases the risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. *Ocular Lesions:* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of O.C.s. Discontinue O.C. medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of certain adenomas, benign or malignant, such as those of the breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently contained in O.C.s, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies have reported an increased relative risk (3.1 to 13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women who took estrogen replacement medication to relieve menopausal symptoms. One publication reported on the first 30 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on O.C.s. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time O.C.s were first given, polycystic ovaries) nearly all occurred in women who had used a sequential O.C. These products are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only O.C.s, although individual cases have been reported. Several studies have shown no increased risk of breast cancer to women taking O.C.s or estrogens. In one study, however, while no overall increased risk of breast cancer was noted in women treated with O.C.s, a greater risk was suggested for the subgroups of O.C. users with documented benign breast disease and for long-term (2-4 years) users. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. A reduced occurrence of benign breast tumors in users of O.C.s has been well documented. In a prospective study of women with cervical dysplasia, there was an increase in severity and of conversion to cancer *in situ* in O.C. users compared with nonusers. This became statistically significant after 3 to 4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to be predictive of progression after prolonged exposure. There have been other reports of microglandular hyperplasia of the cervix in users of O.C.s. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with O.C.s. Close clinical surveillance of all women taking O.C.s is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with O.C. use. This has been reported in short-term as well as long-term users of O.C.s. One study reported that use of O.C.s with high hormonal potency and age over 30 years may further increase a woman's risk of hepatocellular adenoma. Two studies relate risk with duration of use, the risk being much greater after 4 or more years of use. Although it is an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may clearly show a focal defect. Hepatic arteriography may be a useful procedure in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that O.C.s further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. Furthermore, a high percentage of women exposed to diethylstilbestrol (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported following use of sex hormones, including O.C.s, in pregnancy. In one case-control study it was estimated that there was a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (O.C.s, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including O.C.s, during early pregnancy occurred at a rate of 18.2 per 1,000 births, compared to 7.8 per 1,000 for children not so exposed *in utero*. These results are statistically significant. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing O.C.s. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping O.C.s is unknown. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if progestogen-only O.C.s are used) and further use of O.C.s should be withheld until pregnancy has been ruled out. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue O.C.s with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of

another study an increased risk was apparent between 6 and 12 months of use. 7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on O.C.s. For this reason, prediabetic and diabetic patients should be carefully observed while receiving O.C.s. An increase in triglycerides and total phospholipids has been observed in patients receiving O.C.s. The clinical significance of this finding is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with O.C. use. In some women, hypertension may occur within a few months of beginning O.C. use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in O.C. users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, a history of toxemia or elevated blood pressure during pregnancy, a familial tendency to hypertension or its consequences, or a history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevation of blood pressure when given O.C.s and, therefore, should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, the clinical implications of elevations should not be ignored and close surveillance is indicated, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after discontinuation of the O.C. 9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of O.C.s and evaluation of the cause. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses are frequent reasons for patients discontinuing O.C.s. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an O.C. with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of O.C.s. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased prevalence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing O.C.s in comparison with those discontinuing other methods of contraception. The impairment appears to be independent of the duration of use of the preparations. While the impairment diminishes with time, there is still an appreciable difference in the results in nulliparous women for the O.C. and non-O.C. groups 30 months after discontinuation of birth control. For parous women the difference is no longer apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only O.C. failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving O.C.s, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* O.C.s given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in O.C.s has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast fed child have not been determined. If feasible, the use of O.C.s should be deferred until the infant has been weaned. **PRECAUTIONS** *GENERAL* 1. A complete medical and family history should be taken prior to the initiation of O.C.s. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, O.C.s should not be prescribed for longer than 1 year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking O.C.s should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. 4. O.C.s may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving O.C. therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. O.C. users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is unknown. 8. Serum folate levels may be depressed by O.C. therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping O.C.s, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of O.C. therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing O.C.s. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. *DRUG INTERACTIONS* O.C.s may be rendered less effective by virtue of drug interaction with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, and antimigraine preparations. O.C.'s may alter the effectiveness of other types of drugs, such as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., quanethidine), vitamins and hypoglycemic agents. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **ADVERSE REACTIONS** An increased risk of the following serious adverse reactions has been associated with the use of O.C.s (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving O.C.s and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening) change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors. The follwing adverse reactions have been reported in users of O.C.s, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function. **INFORMATION FOR THE PATIENT** (See Patient Package Insert)

Serum sex hormone–binding globulin capacity and the percentage of free estradiol in postmenopausal women with and without endometrial carcinoma

A new biochemical basis for the association between obesity and endometrial carcinoma

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A significant elevation (mean ± SEM) in the percentage of free estradiol was observed in the serum of postmenopausal (PMP) patients with endometrial carcinoma (1.92 ± 0.13%, $n = 13$) when compared with a reference group of PMP women (1.65 ± 0.07%, $n = 29$, $p < 0.05$). This difference was probably due to a lower sex hormone–binding globulin (SHBG) capacity in PMP patients with endometrial carcinoma (26 ± 3 pmol/ml, $n = 20$) than in the reference PMP women (35 ± 2 pmol/ml, $n = 58$, $p < 0.02$), since highly significant inverse correlations between serum SHBG capacity and percentage of free estradiol were found in both groups. Postmenopausal patients with endometrial cancer had a greater mean excess body weight (+69 ± 13 pounds, $n = 20$) when compared to the reference PMP women (+25 ± 4 pounds, $n = 58$, $p < 0.001$), and it was found that serum SHBG capacity was inversely related to excess body weight. Furthermore, when a group of PMP patients with endometrial carcinoma ($n = 11$) were individually matched with PMP reference subjects for deviation from ideal body weight, no differences in SHBG capacity and percentage of free estradiol were observed. Thus, in addition to the well-documented higher than normal peripheral production of estrogen associated with obesity, it also appears that higher concentrations of free estradiol are available to target cells because of a depression in serum SHBG capacity in obese postmenopausal women. These findings provide further evidence that endogenous estrogens may account for the epidemiologic link between obesity and the increased incidence of postmenopausal endometrial cancer. (AM. J. OBSTET. GYNECOL. 138:637, 1980.)

IN POSTMENOPAUSAL women, the ovaries no longer produce significant amounts of estrogen or progesterone. Estrogen is produced largely as a result of the conversion of adrenal androstenedione to estrone in peripheral tissues, and most of the circulating estradiol is produced through the further metabolism of es-

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This work was supported by National Institutes of Health Grant HD 08692.

Presented at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980.

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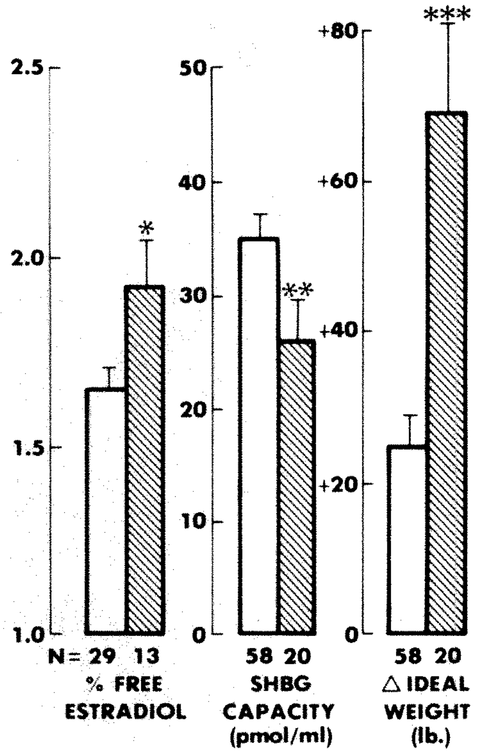

Fig. 1. The mean (±SEM) values of the percentage of free serum estradiol, serum SHBG capacity, and Δ ideal weight of patients with postmenopausal endometrial carcinoma (hatched columns) and postmenopausal reference subjects (open columns). N = Number of subjects in each group. Significant differences between these groups was found in the mean percentage of free estradiol (*), SHBG capacity (**), and Δ ideal weight (***). $^*p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.001$.

trone.[1] Although the concentrations of estrone in the blood are two to three times higher than those of estradiol in postmenopausal women,[2] estradiol appears to be approximately seven times more potent at the level of receptor binding in human tissues.[3, 4] In serum, unconjugated estradiol exists mainly bound to sex hormone-binding globulin (SHBG) and albumin, and only a small (1% to 3%) fraction is unbound. It is generally agreed that only nonprotein-bound (i.e., free) steroid hormones are biologically active, and that the serum SHBG capacity regulates the amount of free estradiol.[5] In view of the relationship between estrogen and endometrial carcinoma, we investigated the SHBG capacity and the percentage of free estradiol in serum of women with and without endometrial carcinoma. Moreover, because of the association between endometrial carcinoma and obesity,[6-9] and previous findings that obesity is related to a decrease in serum SHBG capacity,[10, 11] we paid particular attention to the relationship of serum SHBG capacity to the weight of the patients who were studied.

Patients and methods

Twenty postmenopausal women with endometrial carcinoma and 58 normal postmenopausal women were included in this study. All women were at least 2 years beyond the cessation of menses and gave no history of recent intake of estrogen, thyroid, or other medications known to influence serum SHBG capacity. Samples of serum were stored at −20° C.

The percentage of free estradiol was determined by a centrifugal ultrafiltration-dialysis technique at 37° C, on samples of undiluted serum.[12] Samples (450 μl) were incubated (30 minutes at 37° C) with 3×10^5 dpm [^{3}H]-estradiol and 12×10^3 dpm [^{14}C]-glucose. Duplicate aliquots (200 μl) of the incubates were centrifuged (3,000 × g for 1 hour at 37° C) through a dialysis membrane. The percentage of free steroid in the samples was estimated by comparing the ratio of [^{3}H]-estradiol:[^{14}C]-glucose in the ultrafiltrate with the corresponding ratio in 30 μl of the serum retained by the dialysis membrane.

Serum SHBG capacity was measured in a diethylaminoethyl (DEAE) filter assay similar to that previously described.[13] Samples of serum (50 μl) were diluted 200-fold with the use of 0.01M Tris EDTA (1.5 mM) buffer (pH 7.4 at 21° C), and 300-μl aliquots were incubated (1 hour at room temperature +30 minutes at +4° C) with 10nM [^{3}H]-5α-dihydrotestosterone with and without 100-fold excess of unlabeled 5α-dihydrotestosterone. Duplicate aliquots (100 μl) of the incubation mixture were pipetted onto the DEAE filter discs, which were then washed ten times with 1 ml of the Tris EDTA buffer at 4° C. The amount of [^{3}H]-5α-dihydrotestosterone specifically bound to the SHBG absorbed to the DEAE filters was calculated by subtracting the amount bound in the presence of excess steroid (nonspecific binding) from that bound in the presence of labeled steroid alone (total binding). If it is assumed that SHBG has only one binding site per molecule, the binding capacity could, therefore, be calculated directly.

In each assay, individual samples both from patients with endometrial carcinoma and from reference subjects were included. Sera from two reference subjects, representing high (2.32%) and low (1.37%) percentages of free estradiol and correspondingly low (19 pmol/ml) and high (64 pmol/ml) SHBG capacities, were included in every assay in order to monitor assay performance. The interassay coefficients of variation of these samples (n = 7) were 6.7% and 10.0% for the estimation of percentage free estradiol, and 9.3% and 12.3% for the determination of serum SHBG capacity.

Deviation from ideal body weight (Δ ideal weight) was calculated by means of a standard formula for ideal

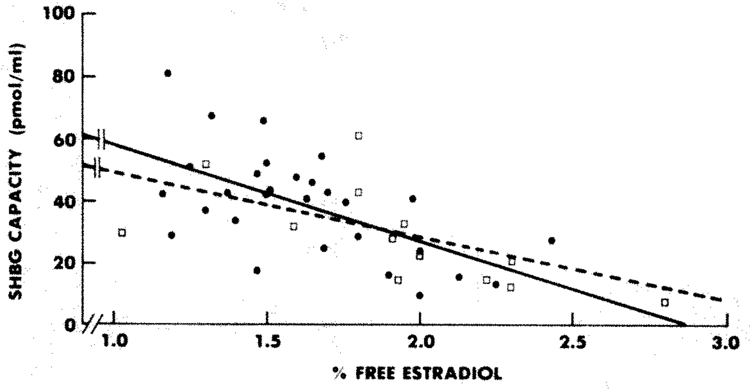

Fig. 2. The relationship between the percentage of free estradiol and SHBG capacity in the serum of 13 postmenopausal patients with endometrial carcinoma (□, broken regression line) and 29 postmenopausal reference subjects (●, solid regression line). A significant correlation was observed both for the postmenopausal patients with endometrial carcinoma ($r = -0.63$, $p < 0.001$) and the reference population ($r = -0.60$, $p < 0.001$). There was no significant difference between the regression lines generated by these two groups.

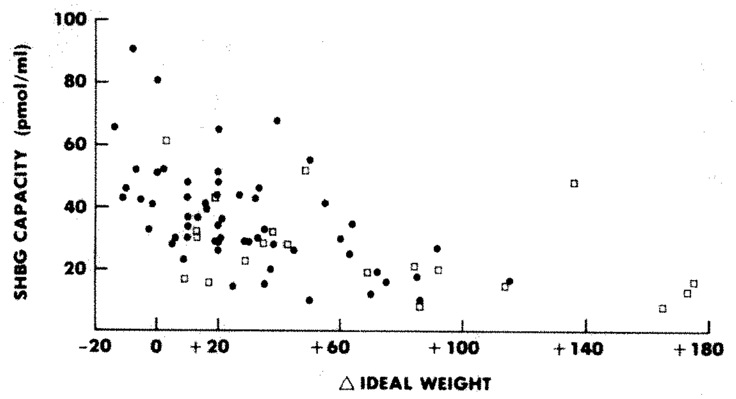

Fig. 3. The influence of Δ ideal weight on the serum SHBG capacity of 20 postmenopausal patients with endometrial carcinoma (□) and 58 postmenopausal reference subjects (●).

weight (Δ ideal weight = $105_{lbs} + 5\ (height_{ins} - 60)$.[14] A two-tailed Student's t test (paired and unpaired where applicable) was employed for statistical analysis.

Results

The mean (±SEM) serum percentage of free estradiol, serum SHBG capacity, and Δ ideal body weight for PMP patients with endometrial carcinoma and reference subjects are presented in Fig. 1. The mean percentages of free estradiol in the sera of 13 patients with endometrial carcinoma (1.92% ± 0.13%) and the 29 reference subjects (1.65% ± 0.07%) were significantly different ($p < 0.05$). The mean serum SHBG capacity of patients with endometrial carcinoma (26 ± 3 pmol/ml, $n = 20$) was significantly lower ($p < 0.02$) than that of the reference subjects (35 ± 2 pmol/ml, $n = 58$), whereas the Δ ideal weight of patients with endometrial carcinoma (+69 ± 13 pounds, $n = 20$) was greater ($p < 0.001$) than that of the reference group (+25 ± 4 pounds, $n = 58$). The mean ages and heights of the patients with endometrial carcinoma (61.1 years and 62.6 inches) were not significantly ($p > 0.05$) different from those of the reference group (58.2 years and 63.6 inches).

The relationship between percentage of free estradiol and SHBG capacity is illustrated in Fig. 2. A highly significant inverse correlation was observed both for the patients with endometrial carcinoma ($r = -0.63$, $p < 0.001$) and for the reference subjects ($r = -0.60$, $p < 0.001$). However, there was no significant difference between the regression lines derived for the endometrial carcinoma and reference groups.

The influence of excess weight on the serum SHBG capacity of patients with endometrial carcinoma and reference subjects is illustrated in Fig. 3 and Fig. 4. The relationship appears to be curvilinear (Fig. 3), but when the SHBG capacity is expressed as the natural logarithm (Fig. 4), a highly significant inverse correlation is

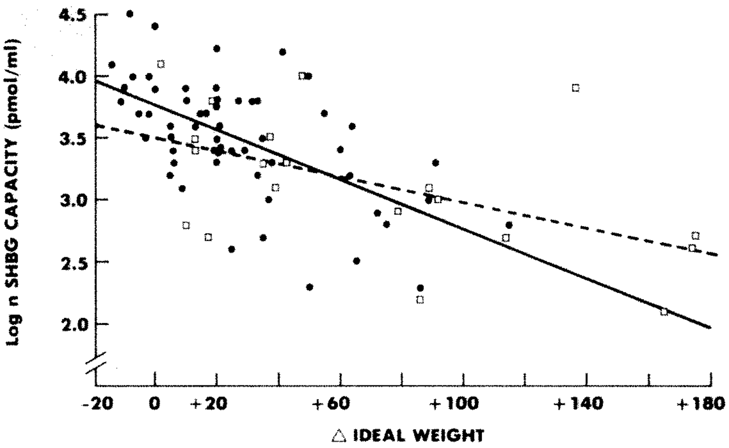

Fig. 4. The relationship of Δ ideal weight to the Log n serum SHBG capacity of 20 postmenopausal patients with endometrial carcinoma (□, broken regression line) and 58 postmenopausal reference subjects (●, solid regression line). A significant correlation was observed both for the patients with endometrial carcinoma ($r = -0.54$, $p < 0.001$, $n = 20$) and for the reference subjects ($r = -0.64$, $p < 0.001$, $n = 58$). There was no difference between the linear regression lines generated by these two groups.

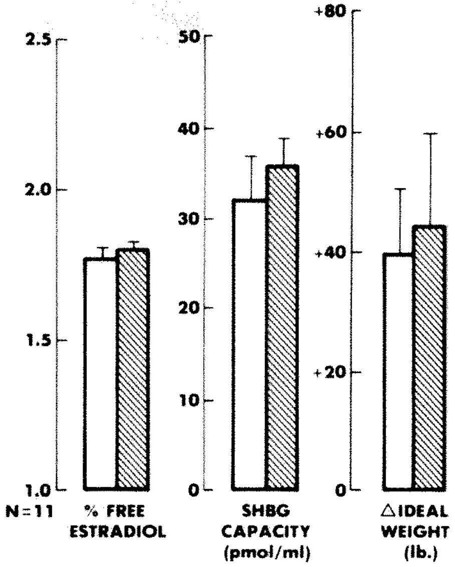

Fig. 5. The mean (±SEM) values of the percentage of free serum estradiol, serum SHBG capacity, and Δ ideal weight of 11 postmenopausal patients with endometrial carcinoma (hatched columns) individually matched with 11 postmenopausal reference subjects (open columns) for Δ ideal weight. No significant differences between these groups were found.

observed both for the patients with endometrial carcinoma ($r = -0.54$, $p < 0.001$) and for the reference subjects ($r = -0.64$, $p < 0.001$). No significant difference was found when the slopes of these regression lines were compared.

In order to further assess the influence of excess weight, a subgroup of 11 patients with endometrial carcinoma were individually matched for Δ ideal body weight with 11 reference subjects. When the estimations of mean serum SHBG capacity and percentage of free estradiol were again compared, no significant difference was observed between patients with endometrial carcinoma and normal women (Fig. 5).

Comment

It was previously demonstrated that the SHBG capacity regulates the percentage of nonprotein-bound testosterone and estradiol in plasma, even though the binding affinity of estradiol is lower than that of testosterone.[5] More recently, Vigersky and associates[15] claimed that estradiol does not bind to SHBG under physiologic (i.e., at 37° C) conditions. However, the latter workers used diluted plasma for their experiments, thus vitiating their conclusion. The present data, which show a strong inverse correlation between SHBG capacity and the percentage of free estradiol when measured in undiluted serum at 37° C, provide strong support for the importance of an estradiol-SHBG interaction in vivo. Moreover, the linear regression lines relating serum SHBG capacity and percentage of free estradiol were very similar for the patients with endometrial carcinoma and normal postmenopausal women, thus suggesting that there is no difference between the SHBG-estradiol interaction in these subject groups. In contrast, we have recently observed that patients with breast cancer have elevated percentages of free estradiol which are not related to a decrease in serum SHBG capacity.[16] The latter observation has been interpreted to suggest that additional factors may interfere with the SHBG-estradiol interaction in patients with breast cancer.

Several factors are known to be associated with a decrease in serum SHBG capacity. These include elevated concentrations of serum androgen, hypothyroidism, and obesity,[5, 10] and of these, only obesity has been consistently found in patients with endometrial carcinoma.[6-9] A direct association has been shown to exist between the relative risk of endometrial carcinoma and the extent of excess body weight,[6] and women who are more than 50 pounds overweight have been reported to develop endometrial carcinoma two[8] to ten[9] times more frequently than women of normal weight. Furthermore, other conditions associated with an increased risk of endometrial carcinoma, such as diabetes mellitus[17] and hypertension,[9] may be risk factors only because they are frequently found in obese women.[18] Although previous studies have suggested that an increased peripheral production of estrogen in obese subjects may account for the relationship between obesity and endometrial cancer,[1] no difference in either the extent of conversion of androstenedione to estrone[19] or serum estrone and estradiol levels[2] has been found between patients with endometrial carcinoma and weight-matched controls. Nevertheless, chronic stimulation of estrogen, unopposed by progesterone, appears to promote the development of endometrial carcinoma, since an increased incidence of this tumor is observed in women with estrogen-secreting neoplasms,[20] anovulatory cycles,[21] polycystic ovarian disease,[22] delayed menopause,[7] and a history of ingestion of estrogen.[23, 24]

The present data demonstrate that serum SHBG capacity is inversely related to excess body weight both in patients with endometrial carcinoma and in reference subjects. The curvilinear nature of this relationship reveals that serum SHBG capacity is markedly reduced in virtually all postmenopausal women who are more than 70 pounds over their ideal weight. Moreover, the linear regression lines generated from the natural logarithm of SHBG capacity and Δ ideal body weight were identical both for patients with endometrial carcinoma and for reference subjects. These data suggest that the lower mean SHBG capacity observed for patients with endometrial carcinoma appears to be due solely to their greater degree of obesity as compared to normal women.

In the serum of patients with endometrial carcinoma, the mean percentage of free estradiol was higher than that in normal postmenopausal women. However, this increase in the mean percentage of free estradiol is probably a reflection of the lower serum SHBG capacity in the patients with endometrial carcinoma, due to their greater mean excess body weight. This is substantiated by the fact that no difference in the percentage of free estradiol in serum could be demonstrated when patients with endometrial carcinoma and normal postmenopausal women were individually matched for ideal weight. Thus, obesity results in an increase in the peripheral production of estrogen,[19] an increased serum concentrations of estrone and estradiol,[2] a decrease in serum SHBG capacity, and a concomitant increase in the percentage of free estradiol in postmenopausal women. These changes, when considered together, suggest that the absolute amount of free estradiol in serum which is available for uptake by target cells is chronically elevated in obese postmenopausal women. Other factors (genetic, viral, etc.) that promote malignant transformation of the endometrium of nonobese women, and that of some obese women but not that of others, remain to be identified.

The observation of decreased serum SHBG capacity in obese postmenopausal subjects is difficult to explain. In addition to obesity, hyperandrogenism and hypothyroidism are known to be associated with a depressed serum SHBG capacity.[5] Although there is little evidence to suggest that the production of androgen is elevated in obesity, adrenal secretion of cortisol and, presumably, adrenal androgens is increased in obese subjects.[25] Similarly, abnormalities in the production or action of thyroid hormone have not been discerned in obesity, despite extensive investigations. However, it is possible that reduced hepatic SHBG synthesis reflects a defect in the action of thyroid hormone in the liver that is not detected by the usual tests of thyroid function. In this regard, it is of interest that the administration of small amounts of thyroxine to obese subjects for relatively short periods of time restores their serum SHBG capacity to normal.[26] Thus, measurement of serum SHBG capacity may serve as an exquisitely sensitive indicator of thyroid hormone function. Furthermore, some of the known interactions between thyroid and sex steroid hormones may be mediated by changes in serum SHBG binding capacity rather than at the cellular level. Indeed, the fact that the administration of thyroid hormone to women with menorrhagia often eliminates the impact of excessive estrogen on the endometrium may be explained by a decrease in the percentage of free estradiol, as a result of increased SHBG synthesis.

The present data provide a biochemical basis for the association between obesity and endometrial cancer. Since a reduction in weight in postmenopausal women has been observed to normalize serum concentrations of estradiol and SHBG capacity,[11] it is suggested that obese postmenopausal women should be advised to lose weight, since a reduction in weight will undoubtedly decrease the amount of biologically active serum estradiol. In addition, the deleterious effects of chronic stimulation of the endometrium by estrogen in obese postmenopausal women should be monitored by periodic sampling of the endometrium. Moreover, excessive estrogenic influence may be reduced by intermittent progestin therapy, which reduces estrogen receptor titers[27] and induces endometrial shedding upon its withdrawal.

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Apparent immunologic nonidentity of human placental and endometrial 17β-estradiol dehydrogenase

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Immunohistochemical techniques were used to search for the presence of 17β-estradiol dehydrogenase activity in human endometrial and placental tissues, with the use of antibodies raised against highly purified human placental 17β-estradiol dehydrogenase. Sensitivity and specificity of the antibodies were documented by radioimmunoassay and immunodiffusion on cellulose acetate. Although staining was consistently demonstrated in the syncytiotrophoblast layer of term placentas, in both cytoplasm and nuclei, no immunohistochemical reaction was observed in endometrial samples. These results support the contention that placental 17β-estradiol dehydrogenase is immunologically dissimilar from the endometrial enzyme. (AM. J. OBSTET. GYNECOL. 138:643, 1980.)

HUMAN ENDOMETRIUM and placenta have been shown to possess 17β-estradiol dehydrogenase (E_2DH) activity. The specific activity of the endometrial enzyme varies during the menstrual cycle. It is low in the follicular phase and increases drastically (over tenfold) after ovulation, reaching a maximum at the midluteal phase.[1, 2] The activity in midsecretory endometrium is about one thirtieth of the level in term placentas, measured under identical in vitro assay conditions.[3]

The EDH activity was demonstrated histochemically only in secretory endometrial glands.[4]

Pollow and coworkers[2, 5, 6] have studied the intracellular distribution of the endometrial enzyme, partially purified it, and determined some of its kinetic characteristics.

The physiologic relevance of changes in E_2DH as a mechanism of regulation of the concentration of E_2 in the endometrium has been emphasized previously.[7] In vivo and in vitro studies have indicated that progesterone and synthetic progestins are inducers of E_2DH.[8]

Human placental E_2DH has been extensively purified and characterized[9–15] and its subcellular distribution has been evaluated.[16] In contrast with the endometrial enzyme, no regulatory mechanisms for placental E_2DH have been reported.

Attempts to neutralize similar levels of E_2DH activity in placental cytosol and in an extract of microsomes with antiserum raised against the purified placental enzyme proved to be successful only for the placental preparation.[3] The lack of cross-reactivity of the endometrial E_2DH with the antiserum suggests immunologic dissimilarity between the endometrial and the placental enzymes.

The present investigation continues the examination of cross-reactivity of human placental and endometrial E_2DH antibodies produced against highly purified placental enzyme.

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Sponsored by the Society for Gynecologic Investigation.

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Material and methods

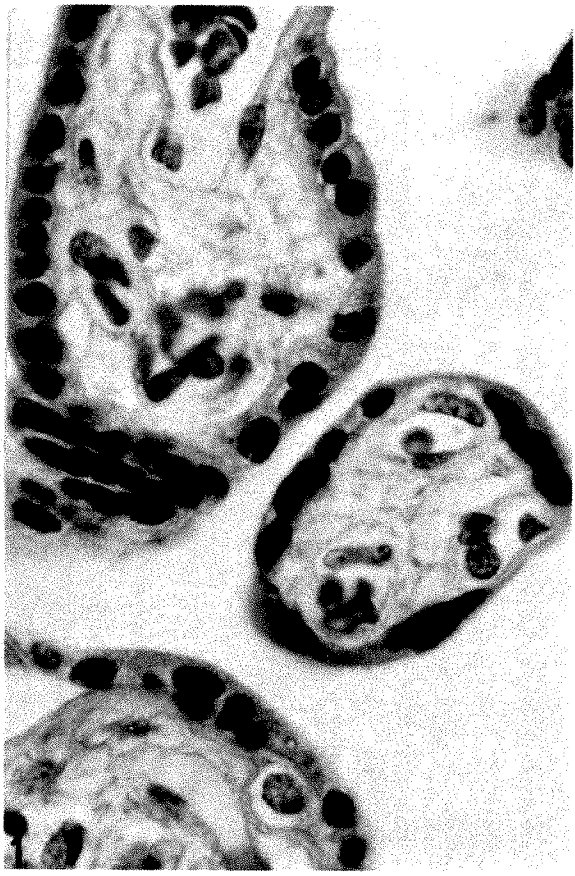
Preparation of antiserum to placental estradiol dehydrogenase. Twelve adult male New Zealand white rabbits were injected with purified enzyme, prepared and supplied by the late Dr. Lewis L. Engel, Harvard Medical School, according to his previously published method.[10] The enzyme was prepared from human placentas and inactivated in the following manner. It was diluted in potassium phosphate buffer, pH 7.2, containing 5 mM ethylenediaminetetra-acetic acid (EDTA), and 50% glycerol to concentrations of 10 to 100 mM. This solution was heated for 30 minutes at 56° C, allowed to cool, and then centrifuged for 10 minutes at 20,000 × g. Injection solutions were made up in 20% glycerol (2 ml), plus complete Freund's adjuvant (6 ml) to a final concentration of 0.125 mg/ml. Initial injections included 0.3 ml intramuscularly in each forelimb, plus 0.2 ml intradermally in three sites on the abdominal wall. Subcutaneous booster injections of 0.2 ml (0.025 mg) were given monthly. Immunologic testing was performed by cellulose acetate immunodiffusion and confirmed with radioimmunoassay by means of techniques previously described.[17] Iodination was by the Chloramine-T method. Although the E_2DH-AB-5 (rabbit 5) was used in this study at a 1:1,000 dilution, the radioimmunoassay was run at a final dilution of 1:200,000. E_2DH-AB-5 was the most active and specific antiserum. The radioimmunoassay was sensitive to less than 2 ng and showed 50% displacement with 22 ng. The curve was linear from 10 to 100 ng.

Lines of identity were obtained on cellulose acetate immunodiffusion between E_2DH-AB-5 and E_2DH in 50% glycerol, 100 mM potassium phosphate, 5 mM EDTA at pH 7.2. No cross-reaction was found with the anti-rabbit gamma globulin added to the serum, and no cross-reaction was found against normal rabbit serum in the same immunodiffusion. Further specificity testing of E_2DH-AB-5 was carried out by means of the radioimmunoassay. No cross-reaction (no displacement of radiolabeled E_2DH) was found with the use of the following compounds: isocitrate dehydrogenase (10 μl of 10 mg/ml), alcohol dehydrogenase from rat liver (100 ng) and from yeast (100 ng), lactic dehydrogenase (10 μl of 10 mg/ml), glucose-6-phosphate dehydrogenase (10 μl of 2 ng/ml), 3,17β-hydroxysteroid dehydrogenase, furnished by Dr. R. Schultz (10 μl containing approximately 100 ng), 3,20-hydroxysteroid dehydrogenase, furnished by Dr. C. Farris (10 μl containing approximately 100 ng), and steroid hormones (progesterone, 100 pg/ml; androstenedione, 28 pg/ml; estradiol, 105 pg/ml; testosterone, 650 pg/ml; and 5α-dihydrotestosterone, 255 pg/ml).

Tissue samples. Samples of histologically proved normal secretory endometrium from surgical specimens and portions of normal, vaginally delivered term placentas were used in these studies.

Immunohistochemical procedures. Tissues were brought to the laboratory in cold (4° C) saline solution, were cut to blocks of appropriate size, and were fixed with cold 2% freshly generated formaldehyde in 0.1M phosphate buffer, pH 7.4. After at least 48 hours in cold fixative, the tissues were dehydrated in alcohol, infiltrated with paraffin, and sectioned (5 to 9 μ) on a rotary microtome. Once adequate morphologic preservation was assured by microscopic examination of routine hematoxylin and eosin preparations, adjacent sections were stained by the immunocytochemical methods of Mason and associates[18] and Sternberger and associates.[19] After deparaffinization and hydration in phosphate-buffered saline solution (PBS), sections were incubated in 3% hydrogen peroxide (5 minutes) and covered dropwise with 1% normal goat serum (NGS). This serum was decanted and replaced by either the specific antiserum (rabbit anti-human placental estradiol dehydrogenase, E_2DH-AB) described above or by various control sera. The dilutions of E_2DH-AB and of the control sera were 1:1,000 (volume:volume). The tissues, covered by antisera, were incubated for at least 24 hours at 4° C in a closed humidification chamber. After this incubation, the sections were washed in three changes (30 minutes each) of PBS, drained, and covered by 1:10 goat anti-rabbit immunoglobulin (30 minutes) at room temperature, washed three times in PBS, and treated with either the Sternberger or the Mason reagents. Those sections to be stained by Sternberger's technique were covered with a solution of peroxidase–anti-peroxidase (PAP) at a dilution of 1:60 in 10% NGS. Tissues processed by the method of Mason and associates were incubated with rabbit anti-horseradish peroxidase (30 minutes), washed in three changes of PBS, and exposed to horseradish peroxidase (Sigma, Type VI) in a concentration of 10 μg/ml of distilled water. Whichever of the above-described immunochemical techniques was used, sections were again washed in three changes of PBS (30 minutes each) before staining with a mixture of 3′5′-diaminobenzidine (DAB) and hydrogen peroxide in Tris buffer. The tissues were washed in distilled water, dehydrated, and covered. Both of these staining procedures gave low, uniform background; the areas of DAB oxidation, indicating localization of specific antigen-antibody complexes, appeared as dark gold-brown to black granules.

Each time the reaction was run, sections were also



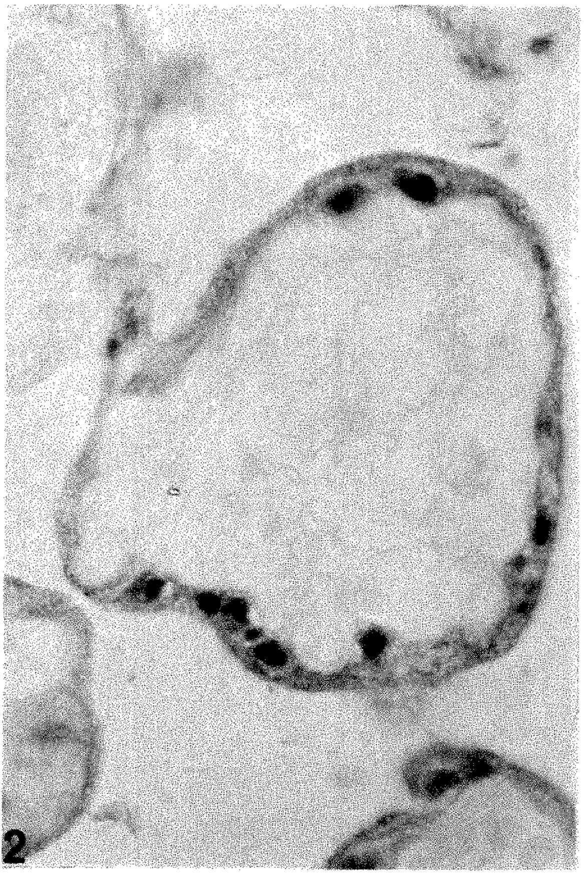


Fig. 1. The normal anatomy of a human placental villus. Note the syncytiotrophoblastic layer surrounding a connective tissue core. (Hematoxylin and eosin. ×750.)

Fig. 2. The villus as prepared by the complete immunohistochemical reaction. Note that only cells of the syncytiotrophoblast show staining. Although nuclei of this cell type are clearly labeled, the cytoplasm and the nuclei of cells at the villar core are not labeled. (Immunohistochemical detection of 17β-estradiol dehydrogenase.) (×750.)

incubated with normal rabbit serum (NRS) or with rabbit antisera prepared against highly purified epidermal growth factor (EGF) and nerve growth factor (NGF); these reagents served as (negative) immunologic controls. Additional sections were only exposed to PBS and then stained with DAB to demonstrate endogenous peroxidase activity and nonspecific staining of the tissue. These tissues were compared with sections similarly prepared but exposed to hydrogen peroxide and normal goat serum as a means of obviating these two effects.

The diluted E_2DH-AB was mixed with highly purified enzyme, supplied by Dr. James Warren, Washington University, St. Louis, Missouri, and incubated at room temperature for 2 hours. Untreated antiserum was allowed to remain under the same condition as a thermal control. Both antisera were used in the staining techniques described above and served as additional immunocytochemical controls.

Results

Placenta. Both immunostaining systems gave virtually identical results when applied to term placenta. The cytoplasm of syncytiotrophoblast showed an appreciable concentration of dark-brown granules, as did each syncytiotrophoblast nucleus (Figs. 1 and 2). Within the villus core, however, cytoplasm and nuclei of all cells consistently failed to stain, thus serving as controls for nonspecific nuclear staining and for regional differences within the sample.

Endometrium. Although convincing and selective placental immunostaining was always demonstrated, specific staining in samples of secretory endometrium was never obtained.

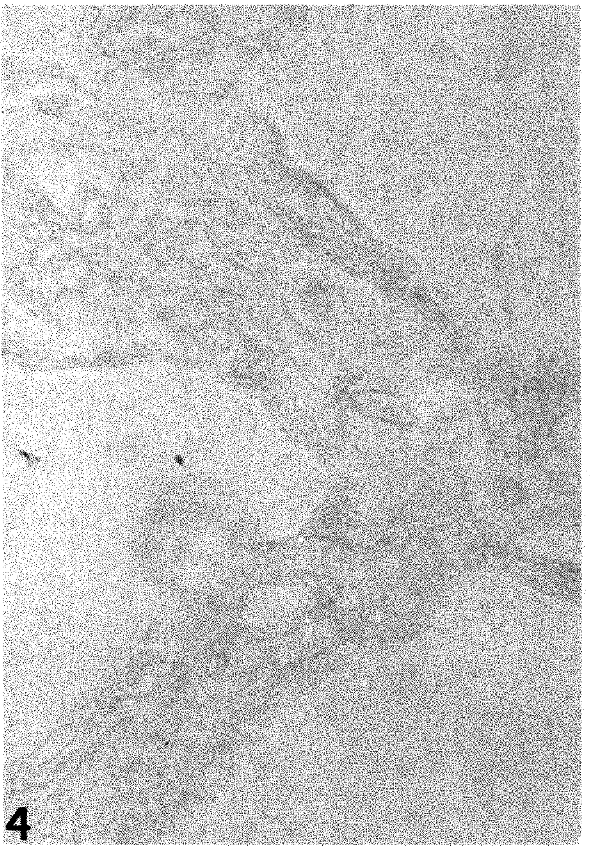


Fig. 3. When another serum (antiectodermal growth factor) was substituted for the specific antiserum as an immunologic control, the placental tissues showed no detectable staining. (Immunologic control.) (×750.)

Fig. 4. The same serum which gave the marked staining reaction documented in Fig. 2 failed to stain the tissue when it was premixed with the purified enzyme. (Immunologic control.) (×750.)

Control. Adjacent sections treated with the full complement of immunologic reagents but lacking either DAB or peroxide showed no staining reaction. Replacement of the specific E_2DH-AB by either NRS (Fig. 3) or by rabbit anti-mouse EGF or NGF did not stain the tissues. When E_2DH-AB was mixed with an excess of the purified placental E_2DH, the staining reaction was abolished (Fig. 4). Unabsorbed E_2DH-AB that was allowed to remain at room temperature for the same period gave positive staining.

The initial treatment of tissues with NGS was a crucial step in revealing specific staining. Without this step, both placenta and endometrium demonstrated intense staining, regardless of which rabbit sera was used. The use of hydrogen peroxide as a blocking agent of endogenous peroxidase activity was a useful adjunct in further reducing background staining. The application of hydrogen peroxide and NGS in concert produced a nearly clear background in controls against which specific staining could be easily and reliably judged.

Comment

It is difficult to rule out the possibility that the negative results obtained with endometrial tissue were due to lack of sensitivity of the method rather than to immunologic differences between the placenta and endometrial dehydrogenase. The latter, however, is the preferred interpretation since histochemical methods, said to be less sensitive than the immunohistochemical procedure used in this work, have revealed the presence of E_2DH in glandular epithelium of secretory endometrium.[4] It should be noted, however, that the sensitivity of the two methods was not formally compared in the present work. Furthermore, differences in the association of the enzymes with particulate subcellular structures in placenta and endometrium[16] can also affect the reactivity of E_2DH with the antibodies.

An intense immunohistochemical staining of nuclei of the syncytiotrophoblast was observed in this study. The reaction was specific, since the nuclei of adjacent placental cells and all nuclei of the endometrium failed to stain. It is not clear whether the reaction was intranuclear or perinuclear, so that the significance of this finding is not known. It is relevant to indicate that the activity of 17β-estradiol dehydrogenase in bovine placenta has been reported to be preponderantly located in nuclei.[20]

The immunologic dissimilarity between the placental and endometrial 17β-estradiol dehydrogenase in women which was suggested by this study indicates that enzymes with the same catalytic function in various tissues may differ structurally and, as a consequence, may vary in their substrate specificity, their affinity to percursors and products in reversible reactions, and their intracellular localization.

The preparation of E_2DH and E_2DH-AB was carried out at the Laboratory for Human Reproduction and Reproductive Biology, Harvard Medical School. In addition to supplying the material, the late Dr. Lewis L. Engel and Dr. E. V. Groman and Dr. H. Lubbert were extremely helpful.

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C-reactive protein as a predictor of infectious morbidity with premature rupture of membranes

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The management of patients with premature rupture of membranes (PROM) poses one of the most serious dilemmas in obstetrics since PROM significantly increases the likelihood of prematurity and serious perinatal infection. Early infection is not reliably predicted nor detected by standard laboratory parameters. Serum C-reactive protein (CRP) levels were assayed along with white blood cell count, differential, and temperature course in patients with PROM and controls. Elevated CRP very accurately divided patients with evidence of infectious morbidity from those without such evidence ($p < 0.001$). In 109 patients there were 11 false negatives and no false positives. In 14 of 20 patients followed with serial comparisons who developed morbidity, CRP became elevated at least 12 hours prior to any other parameter measured. Changes in the other six patients were concurrent. The results suggest that CRP may be a reliable, early predictor of infectious morbidity and thus may be of benefit in the selective management of patients with PROM. (AM. J. OBSTET. GYNECOL. 138:648, 1980.)

THE MANAGEMENT of patients with premature rupture of membranes (PROM) represents one of the most serious dilemmas in obstetric practice. Removal of the barrier to ascending infection dramatically increases the likelihood of the development of chorioamnionitis and generalized fetal or maternal infection. Conservative management, however, has prolonged some pregnancies for as much as several weeks, permitting significant development of the fetus and thus markedly reducing morbidity and mortality due to prematurity.

The ability to detect early infection would allow further maturation of the fetus, yet minimize the sequelae of infection. Early infection is neither reliably predicted nor documented by current laboratory parameters. However, there are certain manifestations of early infection that can be determined in serum and could have clinical application.

C-reactive protein (CRP) was first described by Tillet and Francis[1] in 1930. Lofstrom[2, 3] later detected an "acute phase protein" in serum and identified it as CRP. Further investigation has shown that serum levels of CRP increase with chronic inflammatory processes, infection, and tissue necrosis.[4-6] Recently, Hajj and associates[7] have revived interest in CRP and used a qualitative assay to differentiate inflammatory from noninflammatory gynecologic pathologic conditions.

Although quantitative assays of levels of CRP have been developed in the past 15 years, the techniques often required several days for completion.[8] More recently, accurate methods have been developed (using purified CRP and monospecific antibody) that have allowed much faster results.[9] Thus, the purpose of this study was to assess the predictive value and accuracy of measurements of serum CRP in patients with PROM who were at high risk of developing infectious morbidity.

Method and materials

The study group was comprised of patients who presented to the labor ward of the Chicago Lying-In Hos-

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Supported in part by The Mothers' Aid of The Chicago Lying-In Hospital and Dispensary.

Presented at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980.

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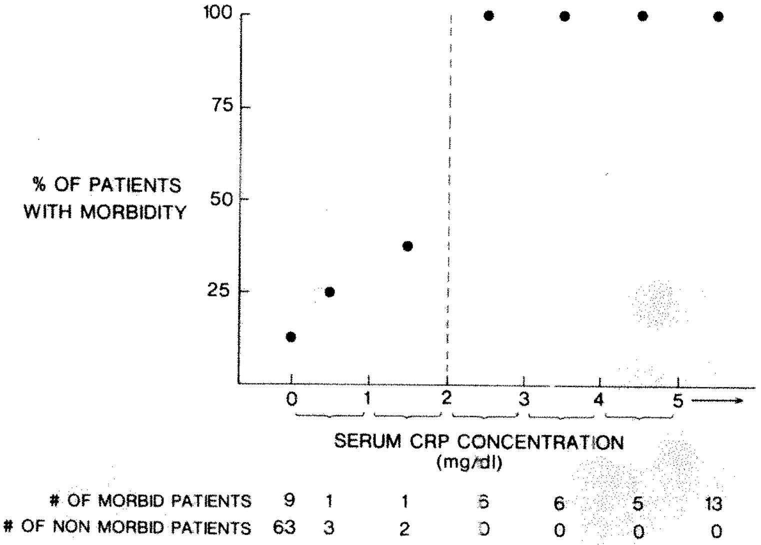

Fig. 1. Percentages of patients with morbidity are defined for given serum concentrations of C-reactive protein. The vertical dashed line represents the arbitrary demarcation point dividing elevated from nonelevated levels of CRP.

pital at 36 weeks' gestation or less and who had a diagnosis of ruptured membranes. Controls were patients with spontaneous rupture of membranes at term (≥37 weeks, SROMAT), preterm labor with intact membranes (≤36 weeks, PTLIM), and term labor with intact membranes (≥37 weeks, TLIM). No patients with intercurrent illnesses, such as systemic lupus erythematosus or rheumatoid arthritis, in which serum levels of CRP are often elevated, were included in the study. Serum levels of CRP, white blood count (WBC), differential, and temperature course were followed serially at 12- to 24-hour intervals in patients who were being managed conservatively. CRP levels were not known to the physicians who were treating the patients during the study.

Infectious morbidity was defined as any of the following antepartum events: temperature ≥37.5° C, maternal or neonatal infection by culture, histologic chorioamnionitis, increase in WBC of 50% over baseline values, more than six bands on differential, or any toxic granulations in white cells. Placental evaluations were performed by attending pathologists unaffiliated with the study.

Quantitative determinations of CRP were performed by means of a nephelometric method with monospecific antisera against CRP and appropriate reference standards (Calbiochem-Behring Corp., La Jolla, California) using the Beckman Immunochemistry analyzer (Beckman Instruments Inc., Fullerton, California).

Statistical analyses were performed wherever appropriate by means of Student's t test and chi-square analyses.

Results

One hundred nine patients were studied from all groups: 36 with PROM, 27 with SROMAT, 6 with PTLIM, and 40 with TLIM. Overall, 41 patients developed evidence of infectious morbidity.

The vast majority of nonmorbid patients (63/68) had no detectable levels of CRP. The other five had levels always less than 2 mg/dl. All 30 patients with elevated CRPs (≥2 mg/dl) had evidence of morbidity. With 2 mg/dl used as an arbitrary demarcation point, CRP divided morbid from nonmorbid patients with great accuracy ($\chi^2 = 65.0$, $p < 0.001$). There were no false positives. There were 11 false negatives which were also not detected by the other laboratory parameters employed. Six patients had histologic chorioamnionitis, four had low-grade fevers (≤37.8° C) without histologic confirmation, and one infant had neonatal infection with beta-streptococcus organisms and did well after antibiotic treatment (Fig. 1).

Divided by categories, CRP levels tended to be higher in patients who also had more than one parameter suggestive of morbidity (Table I). Unfortunately, not all patients or placentas were cultured, so that a few patients who may have had infections were only officially recognized by other parameters. Overall, 97% of all patients with elevated CRP had histologic confirmation of morbidity.

Mean levels of CRP were very signnificantly elevated

Table I. Patterns of morbidity associated with C-reactive protein (No. of patients)

CRP (mg/dl)	*No evidence of morbidity*	*Histologic chorioamnionitis*	*Fever**	*Positive histologic findings, fever,* and/or white cell changes†*	*Positive histologic findings, infection,‡ and/or fever* and/or white cell changes†*
0	63	6	3	–	–
0.1-0.9	3	–	–	–	1
1.0-1.9	2	1	–	–	–
2.0-2.9	–	3	1	2	–
3.0-3.9	–	3	–	3	–
4.0-4.9	–	2	–	1	2
5.0+	–	3	–	7	3

*T ≥ 37.5° C.
†WBC increased by 50% antepartum over baseline values, or >6 bands on differential, or any toxic granulations in white cells.
‡By culture.

Table II. C-reactive protein and other parameters in patients with and without morbidity ($\chi \pm SD$)

Parameter	*All patients*		*p*	*Patients with premature rupture of membranes*		*p*
	Patients with infectious morbidity (n = 41)	*Patients with no evidence of morbidity (n = 68)*		*Patients with infectious morbidity (n = 25)*	*Patients with no evidence of morbidity (n = 11)*	
C-reactive* protein (mg/dl)	4.1 ± 3.4	0.1 ± 0.3	<0.001	4.9 ± 3.4	0.1 ± 0.5	<0.001
White blood count* (10^3 cells/cc)	13.6 ± 3.5	10.0 ± 2.5	<0.05	14.0 ± 3.6	10.4 ± 2.3	<0.001
Bands (per* 100 white cells)	3.6 ± 4.4	1.9 ± 2.7	<0.05	4.5 ± 5.2	0.8 ± 1.0	<0.001
Age (yr)	24.7 ± 6.1	23.9 ± 5.2	NS	25.8 ± 5.1	22.0 ± 2.4	<0.01
Gravidity	3.2 ± 2.0	2.6 ± 1.5	NS	3.8 ± 2.0	3.1 ± 0.9	NS
Parity	1.3 ± 1.6	1.1 ± 1.3	NS	1.8 ± 1.8	0.9 ± 0.8	NS
Gestational age at delivery (wk)	33.5 ± 6.3	37.6 ± 3.8	<0.001	29.8 ± 5.0	32.0 ± 4.4	NS
Fetal weight (grams)	2,145 ± 1,004	2,821 ± 772	<0.001	1,583 ± 789	1,949 ± 661	NS

All p values were obtained by Student's t test. NS = Not significant.
*Values used were highest antepartum levels (all three tests were run on all samples of blood).

in patients with morbidity (4.1 mg/dl) in contrast to those in patients without evidence of morbidity (0.1 mg/dl, $p < 0.001$). WBC and band count on differential also were significantly higher when morbidity was present ($p < 0.05$) but less consistently so than CRP. Fetal weights and gestational ages at delivery were significantly less in the group with morbidity, thus reflecting a higher percentage of premature deliveries in PROM patients than in controls (Table II).

The findings in the subset of patients with PROM who were followed sequentially were very similar to those in the entire group (Table II). The marked elevation of CRP in patients with morbidity was again noticed, along with increased WBC and bands. Of the 36 patients with PROM, 31 were correctly categorized by CRP (Table III). In 14 of 20 patients with PROM who developed morbidity, CRP became positive at least 12 hours before any other parameter. For the other six patients, CRP became elevated concurrently with at least one other parameter.

Not counting CRP, 10 patients with PROM first demonstrated signs of morbidity by changes in the level of one parameter only (elevated WBC, bands, temperature, or the presence of toxic granulations). For this group, CRP levels, which were determined at the same time, gave a 100% predicted rate of morbidity as defined by histologic or culture findings (eight true positives, two true negatives, no false negatives, and no false positives).

Comment

Few obstetric problems have evoked more continuous controversy than the management of premature rupture of membranes. Prior to the advent of powerful broad-spectrum antibiotics, sophisticated intensive-care nurseries, or safe tocolytic agents, PROM posed a major threat to the mother and little chance of survival for the fetus. At present, the ability to treat serious infection, salvage low-birth-weight infants, and stop labor has had an obvious impact on the philosophy of

Table III. Accuracy of C-reactive protein prediction of infectious morbidity

Patient groups	*Positive CRP with morbidity*	*Negative CRP with no morbidity*	*Negative CRP with morbidity*	*Positive CRP with no morbidity*	*Totals*
Premature rupture of membranes (≤36 weeks)	20	11	5*	0	36
Spontaneous rupture of membranes at term (≥37 weeks)	1	23	3	0	27
Preterm labor with intact membranes (≤36 weeks)	1	5	0	0	6
Term labor with intact membranes (≥37 weeks)	8	29	3	0	40
	30	68	11†	0	109

*Two with low-grade fevers (≤37.8° C); three with histologic chorioamnionitis.

†Four with low-grade fevers (≤37.8° C); six with histologic chorioamnionitis; one neonatal infection.

management. At the Chicago Lying-In Hospital a large proportion of the patients are indigent and at high risk for PROM as well as subsequent infection (25/36). Therefore, expectant management of PROM would be quite risky in such a population without a means of detecting early infection.

The results obtained in the study suggest that levels of CRP accurately indicate early infection. Elevated levels of CRP herald a high likelihood of the development of morbidity. In 14 of 20 patients who developed morbidity (and for whom comparisons were possible), elevated CRP measurements were obtained at least 12 hours before any other parameter (temperature, WBC, or differential) suggested infection. There were no false elevated levels of CRP.

It is well known that the standard indicators of infection, including the WBC, differential, and erythrocyte sedimentation rate, are affected in a variable fashion by the menstrual cycle, pregnancy, and especially labor. Margination subsequent to stress causes difficulty in interpretation of an increased WBC. An increased number of band forms, the presence of toxic granulations, or phagocytosed bacteria are more definitive indicators, but often are not reliably detected at early stages of infection. In 10% of the patients studied, CRP was falsely negative, with early, infectious processes that also were missed by all other laboratory parameters as well. Of these 11 patients, six had histologic evidence of chorioamnionitis after delivery, four had low-grade fever without histologic evidence of chorioamnionitis, and one infant had a beta-streptococcal infection that responded well to antibiotic therapy. Although we have included histologic chorioamnionitis as an end point in those cases which were assumed to be positive, one should be aware that the mere presence of leukocytic infiltration need not indicate clinical morbidity. At Chicago Lying-In Hospital, we have found that about one third of all placentas demonstrate leukocytic infiltration. With these statistics in mind, one might reduce further the true incidence of false negatives, and a few false positives might emerge. Such data may reflect samples drawn before significant stimuli were present to effect any changes or may be an inherent limitation of the test. In a few patients with chorioamnionitis, CRP in amniotic fluid could not be detected even after 100-fold concentration. Such data if confirmed by larger sample numbers may confirm that the mother is the sole source of elevated serum CRP.

Assay of serum levels of CRP seems to have certain significant advantages over current methods of detecting infection in PROM. An elevated level of CRP indicated morbidity in 100% of patients in the study. Contrary to the data in previous studies, in which there was incomplete placental evaluation,[10] CRP levels became elevated in labor only when chorioamnionitis or other infectious processes developed. In those cases of PROM in which infection was suggested by only one standard clinical parameter becoming abnormal, CRP predicted histologic evidence of chorioamnionitis with 100% accuracy and thus could serve to alleviate a serious clinical dilemma. In 97% of all patients with elevated CRP, there was histologic confirmation of morbidity. Finally, the data demonstrate the potential usefulness of serum levels of CRP in the diagnosis of early infection.

Current philosophy of management in cases of PROM at the Chicago Lying-In Hospital has evolved toward a "selective conservatism." In gestations of 34 weeks or beyond and/or fetuses estimated to weigh at least 2,000 grams, the hazards of infection usually outweigh those of prematurity in the infant population. Therefore, the group which presents the greatest dilemma consists of fetuses of 26 to 33 weeks' gestation or those who weigh 750 to 1,500 grams, for whom prematurity and its associated cardiovascular-respiratory complications are paramount problems. Prolongation of pregnancy in this group is of obvious benefit when infection can be ruled out or predicted very early in its course. The use of CRP may thus aid in reducing the number of infants delivered unnecessarily because of PROM and allow prompt intervention for those patients who are developing chorioamnionitis.

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The relationship between myometrial activity and sleep state and breathing in fetal sheep throughout the last third of gestation

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Between 106 and 144 days' gestation, periods of uterine electromyographic (EMG) activity and associated increases in uterine tone (contractures) were accompanied by a change in fetal ocular activity in 294 (63%) of 467 cases. In 260 (88.4%) of the changes, there was a switch from the active to nonactive state. The incidence of this change was significantly greater ($p < 0.01$) than the switch from the nonactive to the active state. The fetal electrocorticogram changed state in 34 (74%) of 46 contractures, the change being from low-amplitude to high-amplitude activity in 28 (82%) of the cases. This difference was significant ($p < 0.01$). Fetal breathing movements (FBM), as indicated by activity of the posterior cricoarytenoid muscle, changed in 134 (55%) of 243 contractures, the change being from active to the quiescent state in 115 (85%) of contractures. Diaphragmatic activity changed in 111 (61%) of 183 contractures, with a change from activity to quiescence occurring in 100 instances (90%). Changes in tracheal pressure were associated with an altered state of FBM in 190 (61%) of 314 contractures, and the change was from the presence of FBM to absence of them in 178 (94%) of the changes. In all three of these indicators of FBM, the change from breathing activity to quiescence was statistically significant ($p < 0.01$). (AM. J. OBSTET. GYNECOL. 138:653, 1980.)

INTRAVASCULAR Po_2 has been measured in fetal sheep by means of an indwelling miniaturized Clark electrode.[1] Fetal vascular Po_2 was shown to change continuously in normal preparations over the period of 105 days' gestation to delivery of viable lambs. Slow falls in fetal vascular Po_2 of more than 2 mm Hg were demonstrated to be related to slow changes in basal uterine tone (contractures) that reached at least 3.5 mm Hg above the previously existing intrauterine pressure (IUP) and that lasted at least 5 minutes. The incidence of contractures remained constant over this period at approximately one per hour. These observations led to the hypothesis that rhythmic changes in fetal intravascular Po_2, caused at least in part by alteration in uterine tone, may influence fetal neural activity.[1]

The purpose of the present study was to relate the contractures to two indicators of fetal sleep state, the electro-oculogram (EOG) and electrocorticogram (ECoG), and fetal breathing movements (FBM) as reflected by fluctuations in tracheal pressure (TP) and the electromyographic (EMG) activity of two muscles of inspiration, the diaphragm and posterior cricoarytenoid (PCA). PCA EMG activity has been shown to be a reliable index of FBM.[2] Some of this material has been presented in a preliminary form.[3]

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Supported by Grant 78/3638 from the National Health Medical Research Council and Grant HD 11483-02 from the National Institutes of Health.

Sponsored by the Society for Gynecologic Investigation.

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Table I. Details on experimental animals

No.	*Age at operation (days)*	*Study period (days post-operatively)*	*Hours of record analyzed*	*Outcome of preparation*	*IUP*	*Uterine EMG*	*EOG*	*ECoG*	*Diaphragmatic EMG*	*PCA*	*TP*	*CWD*
1	124	3-20	77	Live fetus delivered at 145 days spontaneously	+		+	+		+	+	
2	126	5-8	60	Live fetus delivered spontaneously at 146 days	+	+	+	+		+		
3	100	6-9	58	Fetus died in utero at 135 days' gestation	+						+	
4	115	14-16	45	Live fetus delivered spontaneously at 141 days	+						+	
5	125	3-9	128	Cortisol-induced delivery at 138 days—live birth	+		+	+		+		
6	118	7-10	6	Live fetus delivered spontaneously at 144 days	+	+	+					
7	115	2-6	97	Fetus alive and well at 131 days*	+		+	+		+		
8	124	8-10	41	Live fetus delivered spontaneously at term	+				+		+	+
9	125	12-14	48	Fetus died during labor at 143 days	+	+	+		+	+	+	
10	116	5-8	65	Fetus alive and well at 130 days*	+	+	+	+		+	+	
11	120	6-25	25	Fetus died during delivery at 145 days	+	+	+		+			
12	113	10-31	†	Obstructed breech delivery at 144 days	+	+	+		+		+	+

*These two fetuses were eventually used for other experiments.
†Fetus 12 was studied solely for the relationship of changes in chest wall dimensions (CWD) and IUP.

Material and methods

Twelve date-mated pregnant Merino ewes at 106 to 144 days' gestation were used for this study (Table I). The ewes were not starved prior to operation, which was conducted with the ewes under 1.5% halothane in oxygen after tracheal intubation. Fetal vascular catheterization was performed at 100 to 125 days' gestation, as previously described.[1] Samples of fetal blood were withdrawn at frequent intervals for the measurement of Po_2, Pco_2, and pH on a Corning blood gas analyzer. An open-tipped catheter (1.52 mm outer diameter, 0.86 mm inner diameter; SV65 Dural plastics) was inserted into the amniotic cavity at the time of operation in order to measure intrauterine pressure.

Measurement of TP. An open-tipped catheter with one side hole (1.52 mm outer diameter, 0.86 mm inner diameter; SV65 Dural plastics) was inserted 6 cm into the trachea through an incision through a tracheal cartilage approximately 2 cm below the thyroid cartilage. The catheter was exteriorized to the ewe's flank, where it terminated in a three-way stopcock. With the stopcock connected to a Statham P23 ID transducer attached to the sheep cage, changes in TP were recorded on a four-channel Grass Model 7D polygraph.

EMG of respiratory muscles. EMGs of two inspiratory muscles, posterior cricoarytenoid and the diaphragm, were recorded with the use of vinyl-insulated multistrand stainless steel wires (Cooner Sales Co., Cat. No. AS632).[2] The wires were sewn into the muscles in such a way that a bare portion of the wire (1.0 to 1.5 mm) remained within the muscle. A pair of wires was introduced 2 to 3 mm apart at each site. An indifferent electrode serving all EMG recordings was inserted subcutaneously over the thorax. The wires, along with the catheters, were then passed through the ewe's flank. The signals were amplified by means of alternating-current coupled amplifiers with high-pass filtering at 80 Hz. The integrated form of the EMGs was displayed on the polygraph by means of a Grass 7P3 amplifier with a time constant of 50 msec.

Access to the PCA was obtained by means of an incision in the inferior pharyngeal constrictor muscle after partial rotation of the larynx. The muscle of the diaphragm was approached through an incision between the tenth and eleventh ribs. The electrodes were introduced 10 to 15 mm from the costal margin.

Recording of ECoG and EOG. The fetal ECoG was recorded from a pair of silver electrodes placed on the dura overlying the parietal lobes, 1.0 to 1.5 cm on either side of the midline. The electrodes were insulated apart from their tips and were cemented into position on the skull by means of cyanoacrylate adhesive. They were connected to stainless steel wire as used in EMG recording. Ocular activity (EOG) was detected

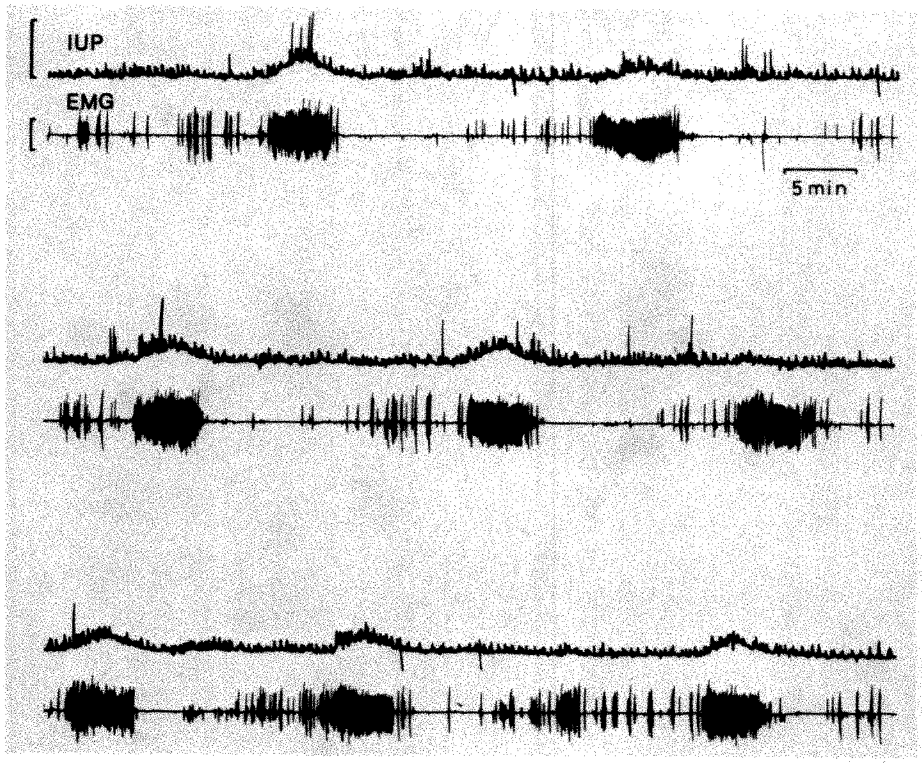

Fig. 1. Relationship of IUP and uterine electromyogram at 115 days' gestation. Calibration bars: IUP, 25 mm Hg; uterine EMG, 1 mV.

from a pair of bared stainless steel wires sewn subcutaneously at the margins of the orbit, above and below the eye.

Recording of uterine EMGs. A pair of EMG electrodes, as described above, was sewn 5 to 8 mm apart into the myometrium of the pregnant horn of the uterus. They were situated on the greater curvature 6 to 12 cm from the tubal extremity. The signals derived from these electrodes were amplified and high-pass filtered at 0.3 Hz. A third electrode that served as an indifferent electrode was placed 2 cm from each electrode.

Recording of chest wall dimensions (CWD). Pairs of ultrasound transducers were attached to the fetal chest in two animals. In one (No. 8), they were placed on the dorsoventral axis at the level of the xiphisternum. In the other (No. 12), they were placed transversely across the chest in the axillary line on each side at the level of the seventh intercostal space. Continuous measurements of the separation between the transducers were made by means of a technique described previously.[4]

Definitions used for scoring the changes in intrauterine pressure (IUP), sleep states, and fetal breathing movements.

Definition of contracture. Contracture is a change in IUP that reached at least 3.5 mm Hg above pre-existing basal pressure and did not return to the previous baseline pressure for at least 5 minutes.

Definition of different EOG states. (+) = Sustained ocular activity; (−) = absence of sustained ocular activity.

Definition of different ECoG states. (+) = ECoG of low frequency and high amplitude; (−) = ECoG of high frequency and low amplitude.

Definition of fetal breathing state from tracheal pressure records. (+) = Movements that produced changes in tracheal pressure consistently greater than 2.5 mm Hg; (−) = no changes in tracheal pressure consistently greater than 2.5 mm Hg.

Definition of fetal breathing state from diaphragmatic or PCA records. Bursts of diaphragmatic or PCA activity were scored individually; therefore, change of state could be directly quantified (see below).

Definition of the four possible state relationships occurring at the time of a contracture.

TP, ECoG, AND EOG RECORDS.

1. (+)/(−); (+) = State that existed for at least 2 minutes prior to a change to (−) state that lasted at least 5 minutes. The change had to occur during the contracture. The short period of 2 minutes was chosen for the original state since this period was considered to be long enough to establish the state; if the period was longer, it could be argued that there would be a natural

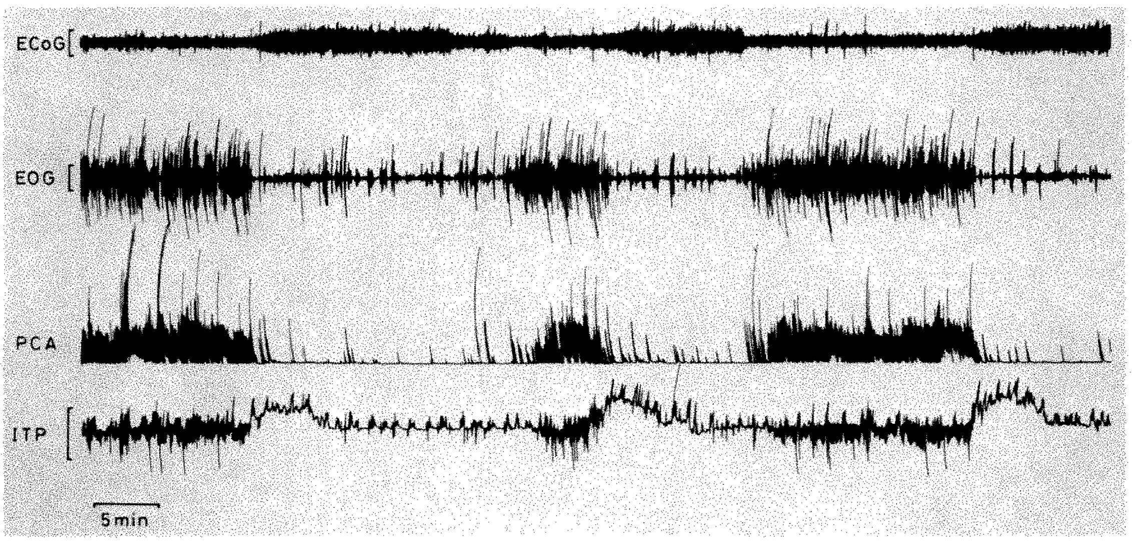

Fig. 2. Relationship of ECoG, EOG, PCA, and ITP at 134 days' gestation. Calibration bars: ECoG, 100 μV; EOG, 100 μV; ITP, 25 mm Hg.

Table II. Incidence of the various state changes for the two sleep states and three indices of fetal breathing movements

	No. of animals studied	*Total No. of contractures*	*(+)/(+)*	*(+)/(−)*	*(−)/(−)*	*(−)/(+)*	*EP*- (mean ± SEM)*	*P*
EOG	8	467	91	260	82	34	0.884 ± 0.019	<0.01
ECoG	2	46	3	6	9	28	0.076 ± 0.065	<0.01
PCA	4	143	49	115	60	19	0.858 ± 0.3	<0.01
TP	6	314	40	178	84	12	0.937 ± 0.018	<0.01
Diaphragmatic EMG	3	183	34	100	38	11	0.901 ± 0.028	<0.01

*The estimated proportion (EP) is the proportion of (+)/(−) changes as designated in Equation 1 in "Materials and methods." allowing a weighting for the sample size.

The probability relates only to the changes in state as discussed in "Material and methods."

tendency for the change to occur spontaneously. The longer period of 5 minutes was chosen for the new state to weight the scoring against chance, short-lived changes influencing our hypothesis positively.

2. (+)/(+); (+) = State that existed before the contracture and continued throughout.

3. (−)/(−); (−) = State that existed before the contracture and continued throughout.

4. (−)/(+); (−) = State that existed for at least 2 minutes prior to a change to (+) state that lasted at least 5 minutes. The rationale for the choice of times was the same as discussed above for the (+)/(−) state change.

DIAPHRAGMATIC AND PCA EMG RECORDINGS. Initial period was scored (+) if there were continuous bursts of diaphragmatic or PCA EMG activity, (−) if the incidence of bursts of diaphragmatic or PCA activity was less than four per minute. A 50% change in the number of bursts of diaphragmatic or PCA EMG activity was scored (+) or (−) at any time during the contracture and lasting at least 5 minutes.

Statistical analysis. As described above, contractures could be accompanied by four different combinations of state of each biophysical variable (+)/(+) and (−)/(−) when the state that existed before the contracture did not change in relation to the contracture, and (+)/(−) and (−)/(+) when the contracture was associated with a change of state. The states (+)/(+) and (−)/(−) represented continuation of state and were not integer values.

We have analyzed only the changes of state, since these were integer values. Under the null hypothesis of their being no association between the change in IUP and the various biophysical activities, it would be expected that any change in the various activities occurring during the contracture would be (+)/(−) or (−)/(+) with equal probability. Therefore, a test of associa-

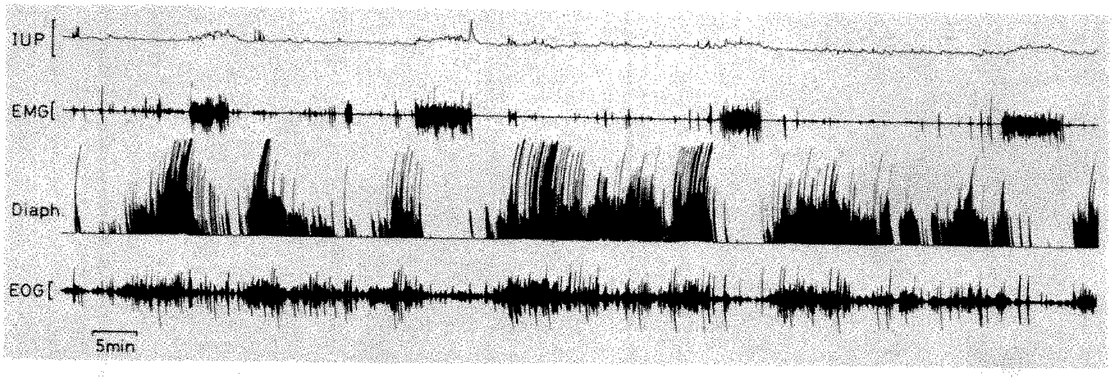

Fig. 3. Relationship of IUP, Uterine EMG, diaphragmatic EMG *(Diaph.)*, and EOG at 126 days' gestation. Calibration bars as for previous figures.

tion would consist of a comparison of the proportion in Equation 1.

$$\frac{[(+)/(-)]}{[(+)/(-)] + [(-)/(+)]} = 0.5. \qquad (1)$$

This method of testing whereby only changes in activity were used was considered to be necessary because of our inability to analyze by eye the proportions of time in each different state over the whole period recorded. Any statistical analysis on the state (+)/(+) or (−)/(−) could only be performed after detailed computer analysis to establish the total time in the various states and a satisfactory weighting of the many intermediate areas in which the actual state was not totally clear.

Results

Animals used and outcome of the various preparations. The details on the 12 animals used are shown in Table I. No studies were performed during the first 48 hours postoperatively, and observations were made 10 to 20 days after fetal catheterization in five animals. Ten ewes were allowed to go into labor; in nine the fetus was alive and well when labor commenced. The fetuses were delivered alive. No observations obtained within 72 hours of fetal death in utero were analyzed. Blood gases were within the normal range (jugular pH 7.35 ± 0.001; Pco_2 42.1 ± 0.7 mm Hg; Po_2 19.0 ± 0.3 mm Hg—Mean ± SEM, n = 85).

Frequency of contractures. In 712 hours of record analyzed in 11 animals there were 675 contractures. The incidence of contractures in individual preparations ranged from 0.5 to 2.0 per hour, with an average incidence of 0.9 per hour.

Relationship of contractures to myometrial EMG. In the six preparations in which uterine EMG leads were applied, contractures were always accompanied by a burst of EMG activity (Fig. 1). The electrical activity consisted of groups of spikes 0.5 to 2 mV in amplitude. Bursts of EMG activity were frequently seen which were not accompanied by an increase in IUP. Therefore, the incidence of bursts of EMG activity was greater than the incidence of contractures as defined above.

Relationship of contractures to fetal sleep state. In 467 contractures in eight animals there was a change in ocular activity (EOG) in 294 (63%). In 260 of these changes (88.4%), the change was from a state in which ocular movements were present (+) to one in which they were absent (−) (Figs. 2 and 3) (Table II). The occurrence of the (+)/(−) change was significantly greater than the (−)/(+) change ($p < 0.01$).

In the two animals in which ECoG was analyzed, there was a change of state in 34 (73.9%) of 46 contractures. The (−)/(+) state change (Fig. 2) occurred significantly more frequently than the (+)/(−) change ($p < 0.01$; Table II).

Relationship of contractures to three indices of fetal breathing. Change of state occurred in 55.1%, 60.7%, and 60.5% of contractures for PCA, diaphragmatic, and TP recordings, respectively (Table II, Figs. 2, 3, and 4). The change during the contracture was (+)/(−) in 85.8%, 90.1%, and 93.7% of these contractures for PCA, diaphragm, and TP, respectively ($p < 0.01$) (Table II).

Relationship of contractures to CWD. In both fetuses into which ultrasound transducers had been implanted, changes in CWD occurred in relation to the contractures. In the fetus with dorsoventrally positioned transducers, CWD showed only reductions of up to 1 cm during many, but not all, contractures (Fig. 4). In the other fetus with bilaterally placed transducers, the distance between the transducers increased by up to 1.5 cm (Fig. 5). The change in the bilateral di-

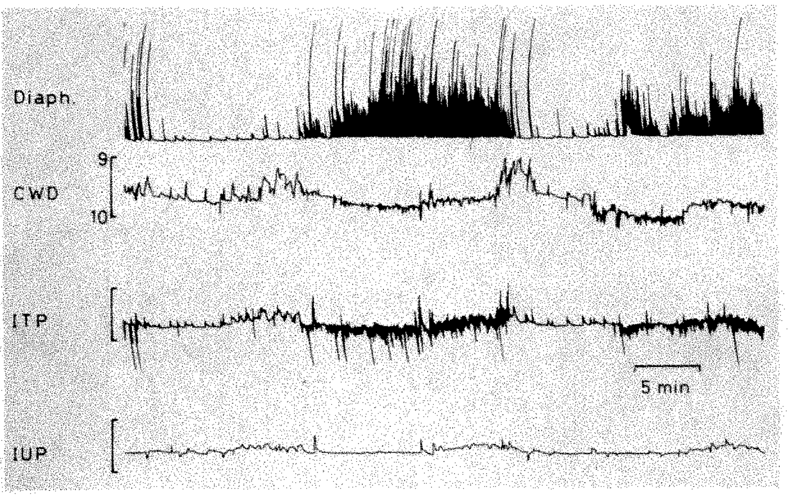

Fig. 4. Relationship of diaphragmatic EMG *(Diaph.)*, CWD, ITP, and IUP at 132 days' gestation. Calibration bars as in previous figures. CWD = distance between dorsal and ventrally placed transducers, in centimeters.

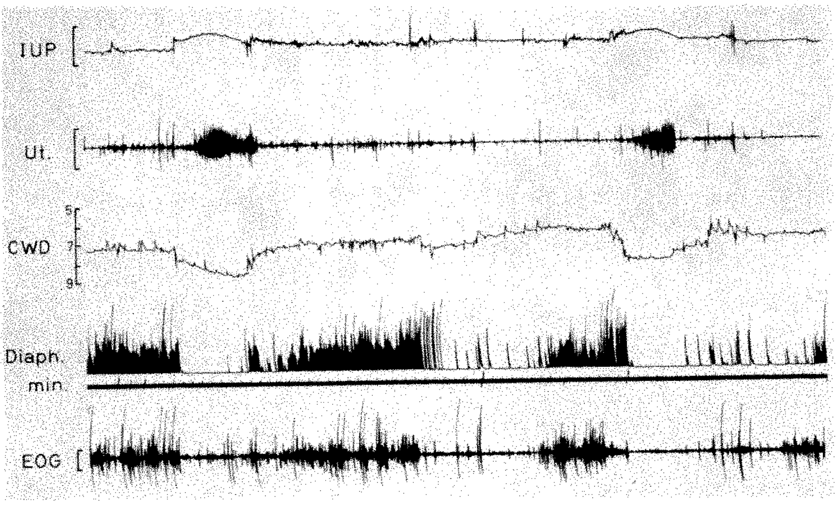

Fig. 5. Relationship of IUP, Uterine EMG *(Ut.)*, and CWD. Diaphragmatic EMG *(Diaph.)* and EOG at 137 days' gestation. Calibration bars as in previous figures. CWD = distance, in centimeters, between bilaterally placed transducers in the midaxillary line at the level of the seventh intercostal space.

mension occurred more consistently than that in the dorsoventral dimension.

Comment

In most studies of the physiology of the sheep fetus in late gestation, IUP has not been recorded and the presence of contractures has gone unnoticed. Whenever they have been noticed, their potential physiologic significance has not been discussed. The causal relationship of these tonic IUP changes to myometrial contractions has been clearly demonstrated by the close relationship of the contractures to electromyographic activity of the uterus. Contractures are, therefore, not caused by other muscular activity, such as movements of the rumen or postural changes. The overall frequency of the contractures in 11 animals over 712 hours was 0.96 per hour. This corresponds well with the incidence of 0.94 per hour in 13 animals over 585 hours reported previously.[1]

The absolute value of IUP measured with either balloon or open-tipped catheters in the pregnant sheep shows considerable variation from animal to animal. Direct observation of the uterus at the time of operation for the implantation of vascular catheters shows that contractures are localized to differing areas of the uterus and occupy varying amounts of myometrium.

Thus, equivalent changes in IUP may represent varying amounts of development of tension in different areas of myometrium. The tip of an open-tipped catheter may become partially blocked by membranes. For these theoretical and methodologic reasons the IUP changes recorded vary from animal to animal, but our experience has been that contractures are present in all pregnant ewes beyond 100 days' gestation. The ability of contractures to produce marked distortion of the fetal thorax (Figs. 4 and 5) illustrates their extent and suggests that they are capable of altering the nature of the sensory input to which the fetal nervous system is exposed.

Passive changes in IUP caused by differences in maternal posture—especially lying down—did not apparently affect fetal sleep state or breathing movements. We previously presented preliminary data that demonstrated a temporal relationship between contractures and high-amplitude, low-frequency ECoG activity.[5] In the present study, we demonstrated that a change in EOG and ECoG states occurred in 63% and 74% of cases, respectively. Although these are high figures, their statistical significance cannot be calculated unless a clear analysis of the period of time spent in each state can be made. Although estimates of the time spent in each state have been made by different authors,[6-9] these reports do not state the detailed methodology used. In our opinion, arbitrary criteria and intricate computer analysis would be necessary to categorize the intermediate "states" adequately enough to permit an analysis based on all four possible combinations present before and during each contracture. The frequency of the two changes in state can, however, be analyzed, and the (+)/(−) and (−)/(+) states for EOG and ECoG, respectively, occur in a significantly higher frequency than would be accounted for by the null hypothesis. Similarly, the proportion of (+)/(−) changes for the three monitors of FBM was significantly different from 0.5. These findings are in agreement with the positive relationship demonstrated between low-amplitude ECoG, activity of the ocular muscles, and FBM.[6, 7]

Variability in the extent of depression of FBM during contractures may reflect differences between animals in the various influences acting on the fetal respiratory center, and differences in the extent of IUP changes associated with the contracture. According to the exact location of myometrial activity, individual contractures may impair cotyledonary blood flow to differing extents. Compensatory changes in the distribution of fetal and maternal blood flow may also influence the final extent of the changes in fetal sleep state and FBM associated with contractures in individual animals. In view of the multiplicity of factors affecting sleep state, the demonstration of such a strong relationship to contracture is remarkable.

We previously suggested that rhythmic changes in fetal intravascular Po_2, caused at least in part by alteration in uterine tone, are able to influence fetal neural activity.[1] The observations reported here demonstrate that contractures are related to fetal sleep state and FBM. Further experiments are necessary to assess the exact temporal and causal relationships. However, the possibility that regular IUP changes constitute a major pathway whereby maternal rhythms affect the fetus remains an intriguing possibility.

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Monolayer and suspension culture of human prolactin-secreting pituitary adenoma

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In order to study the hormonal characteristics of human prolactin-secreting pituitary adenomas, in vitro monolayer and suspension cultures from human pituitary glands were established. Optimal conditions for cultures included enzymatic dispersion into viable single-cell suspensions with the use of 1% collagenase in phosphate-buffered saline solution. After pelleting the dispersed cells by centrifugation (800 rpm for 10 minutes), they were cultured in RPMI medium that contained 20% fetal calf serum and then incubated at 37° C in 5% CO_2. Cells were subcultured weekly at a ratio of one plate to two. In an attempt to establish whether bromocriptine has a direct inhibitory effect on pituitary secretion of prolactin (PRL), variable doses of bromocriptine were added to duplicate plates. The addition of bromocriptine to the culture medium induced suppression of PRL within 7 days. In conclusion, this study demonstrated that either monolayer or suspension cultures of human PRL secreting adenomas can be established, and that bromocriptine in doses of 1 ng/plate or more has a direct inhibitory effect on the secretion of PRL. (AM. J. OBSTET. GYNECOL. 138:660, 1980.)

SECRETION of prolactin (PRL) is controlled primarily by a neuroendocrine mechanism which is inhibitory in nature.[1, 2] This inhibitory control is mediated by dopamine.[3] Bromocriptine, a dopamine agonist, has been shown to inhibit the secretion of PRL.[4] In addition, in organ cultures of rat and human fetal pituitary glands, bromocriptine, in doses ranging between 1 and 5 μg/ml, has been shown to have a direct inhibitory effect on the secretion of PRL.[5] Recently, isolated reports have demonstrated that the administration of bromocriptine to patients harboring PRL-secreting pituitary adenoma did result in an apparent regression of a suprasellar extension of the adenoma.[6-8] This tumor regression has not been determined to be due to either a pituitary or hypothalamic effect. The monolayer and suspension cultures developed in this laboratory from surgically obtained human PRL-secreting adenomas offer a further opportunity for studying this problem. This report illustrates a direct inhibitory effect of bromocriptine on the secretion of PRL from human adenomatous pituitary cells in vitro.

Material and methods

Pituitary adenoma cells were obtained from patients with amenorrhea-galactorrhea and hyperprolactinemia who were undergoing transphenoidal sella exploration for treatment of previously diagnosed PRL-secreting pituitary adenoma. The surgically obtained specimens of human tissue were collected in sterile normal saline solution and transported immediately to the laboratory. Each piece of tissue was thinly minced, placed in 1% collagenase (Worthington) in phosphate-buffered saline solution (PBS), pH 7.2, and mixed until the cells were dispersed. The time necessary for complete dispersion of cells varied between 5 and 20 minutes, depending on the thickness of the individual tissue. After centrifugation (800 rpm for 10 minutes), the cell pellet was resuspended in 4 ml of RPMI medium and placed in culture plates (60 by 15 mm) or flasks (75 cm^2) (Falcon Plastics) at a concentration 1×10^5 cells per plate counted in a hemocytometer. Cell viability, as determined by staining with trypan blue, averaged

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Sponsored by the Society for Gynecologic Investigation.

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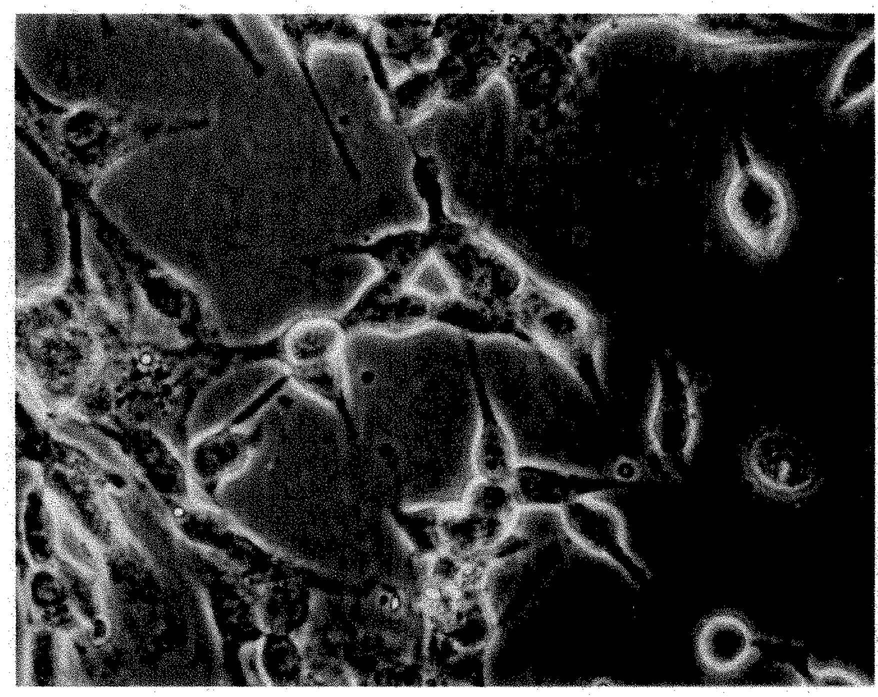

Fig. 1. Phase contrast photomicrograph of an adherent culture of dispersed cells from a human PRL-secreting adenoma. (×400.)

80%. The RPMI medium (Irvine Scientific Co., Irvine, California) was supplemented with 20% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). The dispersed cells were incubated at 37° C in a 5% CO_2 environment. The medium was changed every 4 days, except with experiments in which bromocriptine was used, in which case medium containing bromocriptine was changed daily. When the monolayer culture plates became confluent, they were subcultured, usually on a weekly basis, with 1% collagenase in PBS to disperse cells. The suspension culture was also refed every 4 days and subcultured every 30 days when the medium became very turbid. Cell suspensions were pelleted at 400 rpm for 5 minutes in a table-top centrifuge. The resultant pellet was resuspended in fresh RPMI medium, triturated to disperse the cells, and further equally subcultured into new dishes at a ratio of 1:2, maintaining a constant volume of 4 ml per plate. Cell-free aliquots of spent medium were collected and stored at −20° C until PRL was measured by radioimmunoassay (RIA) as previously reported.[9]

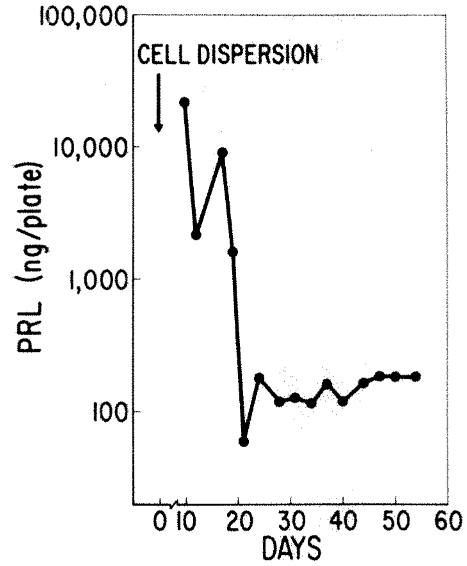

Fig. 2. Mean PRL concentration in medium collected from duplicate dishes of monolayer cultures, 4 ml/plate.

Bromocriptine mesylate (provided by Sandoz Co.) was initially dissolved in 0.024N HCl and 95% ethanol in a 9:1 ratio. Final concentrations were made with culture medium which was then added to the cells on a daily basis. Both monolayer and suspension culture cells were treated with doses of 1, 10, and 100 ng, 1 μg, and 1 mg per plate per day. Control plates were treated only with the vehicle used to dissolve the bromocriptine. The results obtained in two different tissue specimens are reported here.

Results

The physical appearance of human cells from PRL-secreting pituitary adenoma in monolayer culture are

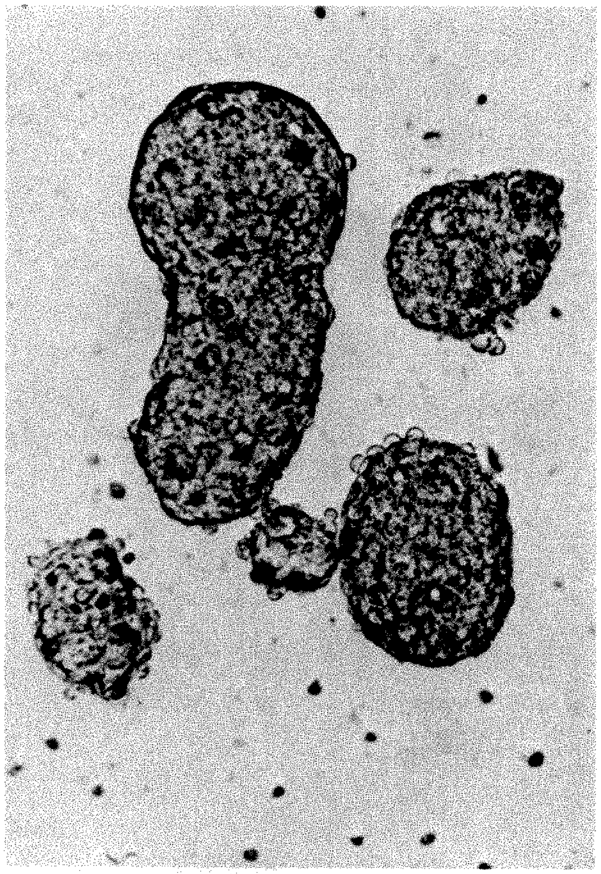

Fig. 3. Photomicroggaph representing a naturally occurring suspension culture of human PRL-secreting pituitary adenoma. (× 100.)

illustrated in Fig. 1. These cells were of irregular shape and adherent to the culture plate. Since these cultures had not been cloned, there was a mixture of epithelial and fibroblast cells. However, a significant number of PRL-secreting cells was initially present, as evidenced by the elevated levels of PRL found in the culture medium (Fig. 2). The level of PRL detected in the medium was noticed to drop over a 3-week period, at the end of which time a plateau of approximately 200 ng/plate was reached. This plateau was maintained for an additional month, thus allowing for sufficient experimental time. After primary dispersion, cells from one specimen remained in suspension and grew in large aggregates which progressively enlarged, resulting in a turbid suspension (Fig. 3). These conglomerates of cells were highly active secretors of PRL, with up to 40,000 ng/plate secreted over 4-day periods (Fig. 4). This suspension culture remained active for more than 60 days.

The experiments with bromocriptine were initiated on first-passage suspension cells with the use of the dosage schedule previously described. The effect of bromocriptine on the release of PRL is shown in Fig. 5. By the third or fourth day of treatment, the concentration of PRL was reduced by 50%, whereas the control plates showed an actual increase in secretion of PRL. Although the control plates showed some decline in the secretion of PRL by the seventh day, the hormone level remained higher than the initial levels. In contrast, plates treated with bromocriptine released significantly less PRL ($P < 0.001$) at the end of the experiment than the controls. Fig. 6 illustrates a dose response with increasing doses of bromocriptine added to the suspension culture.

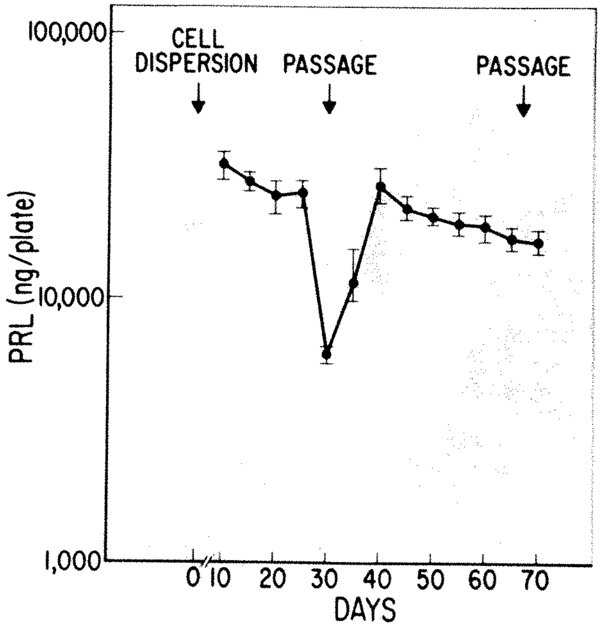

Fig. 4. Mean ± SEM concentration of PRL in medium collected from six dishes of suspension culture, 4 ml/plate.

Comment

The results of this study demonstrate that in vitro maintenance of human PRL-secreting pituitary adenoma is possible either as a monolayer or suspension culture. The PRL released in early stages of culture most likely represents a combination of newly synthesized PRL and the preexisting, internalized PRL present at the time of initiation of the cultures. Although the amount released into the medium varied with the culture system used and the specimen, PRL was found in the cultures for a minimum of 2 months.

The PRL collected over the life span of the cultures ranged up to milligram quantities, which represents an important and significant source of PRL available for numerous experimental purposes. Therefore, the amount of PRL collected from such cultures is sig-

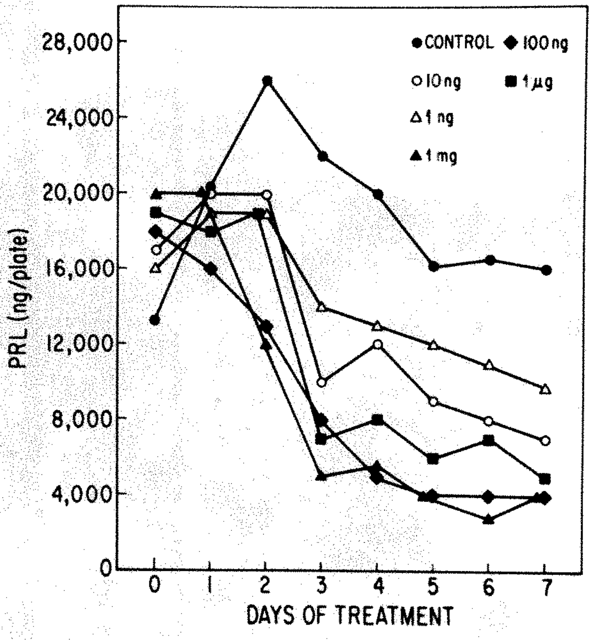

Fig. 5. Effects of bromocriptine on secretion of PRL in suspension culture obtained from human PRL-secreting pituitary adenoma.

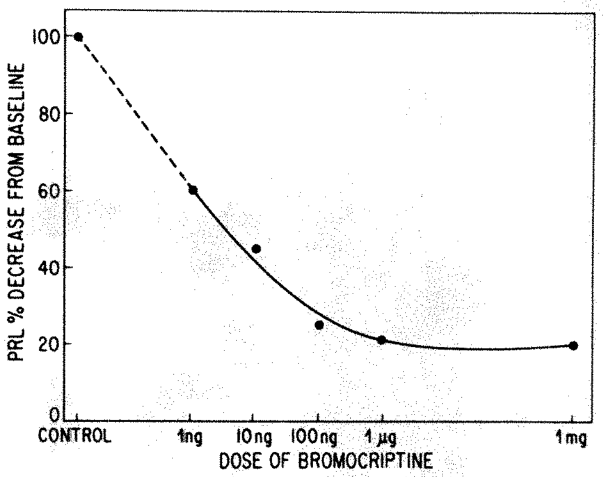

Fig. 6. Secretion of PRL in suspension culture with increasing doses of bromocriptine. (●----● Projected decline from control.)

nificantly greater and less expensive than that obtained from normal human pituitary glands.[10] Our findings of large amounts of secreted PRL are in agreement with those of Skyler and associates[11] of large quantities of PRL and growth hormone (GH) secreted in vitro by fragments of pituitary tumor from an acromegalic patient.

The monolayered cells appeared to be a mixture of both epithelial and mesenchymal elements. As previously reported, after 3 weeks in culture, their capacity to synthesize PRL, or at least to secrete it, gradually diminished, and after 2 months this capacity was markedly reduced.[12, 13] The problem of long-term or even short-term maintenance of human pituitary cells is one inherent in the system, although continuous cultivation of rat pituitary cells has met with greater success.[14] We and others previously noticed that the establishment of in vitro culture of human pituitary adenoma is subject to marked difference in the method of enzyme dispersion, growth medium, and other standard conditions required for the growth and maintenance of these cells.[15, 16] The conditions described for these monolayer cultures allow for at least 2 months of experimentation. This relatively short period of growth may be due to an overgrowth of fibroblasts, or to the lack of specific factors in the medium for maintenance of lactotrophs or synthesis of PRL.

The suspension culture, which spontaneously arose from a primary explant, was capable of secreting high levels of PRL. As indicated in Fig. 4, levels of PRL remained high and relatively constant in suspension cultures for 20 to 30 days. These readings, approximately 30,000 ng/plate, were the result of newly secreted PRL, since the medium was totally replaced every 4 days. This was followed by sharp declines in the secretion of PRL, and the cells were subcultured. Eight days later, levels of PRL were again high, similar to those found prior to decline. This may indicate a need for subculturing at monthly intervals. Whether this line represents a unique transformant of a less adherent cell type is a moot point at present. However, this preliminary finding is of interest as a point of exploration in a complex area. Further experimentation is necessary to determine whether suspension cultures will routinely allow for massive production of PRL, thus eliminating the inconvenience of fibroblasts found in monolayer cultures. A fibroblast-free culture obtained by shaking off loosely attached cells was reported by Kohler and associates.[17] However, these cells did not grow in suspension, and the levels of GH secreted were not clearly stated.

The demonstration that bromocriptine inhibits the release of PRL by a direct effect on the suspension cells is in agreement with previous reports.[5, 18] The degree of inhibition did correlate with the dose of bromocriptine used, since appreciable difference could be demonstrated over the range of doses. This response to dose seen in vitro may be an explanation for the clinical response in which doses of 20 mg per day were apparently sufficient to decrease the size of the tumor.[6–8] Further studies in vitro are necessary to determine the effect of acute versus long-term dose-related treatment on cell structure.

We are grateful to the National Institutes of Health, Bethesda, Maryland, for the generous gift of reagents used in the PRL assay. We also wish to thank Ms. Lillian Goedert for her technical assistance.

Addendum

Since the completion of the present study, we were able to maintain in suspension six additional human PRL- and growth hormone–secreting pituitary adenomas for up to 12 weeks.

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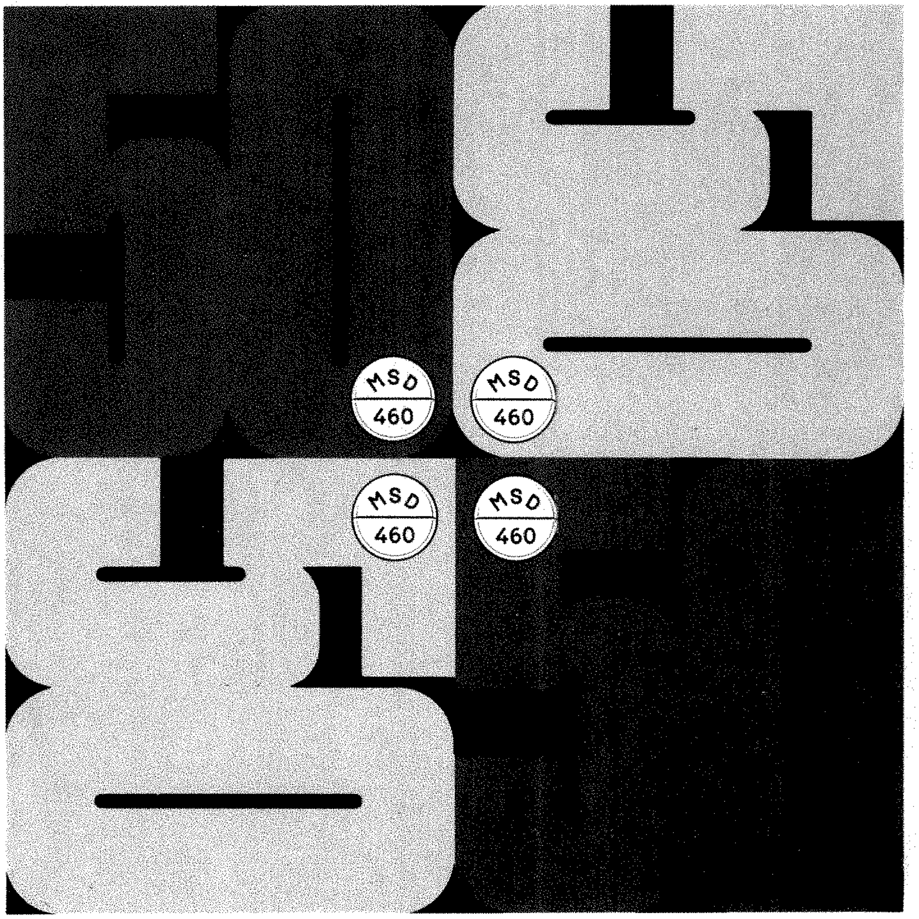

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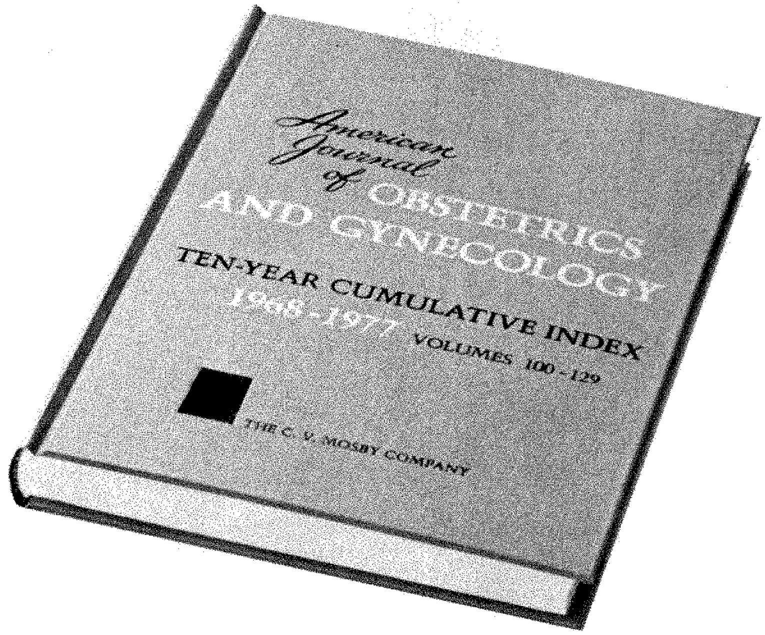
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Stimulatory activity of serum on prolactin production by human decidua

ANTHONY A. LUCIANO
ILA A. MASLAR
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Farmington, Connecticut

Fetal calf serum (FCS) enhances the synthesis and secretion of prolactin (PRL) by explants of human decidua incubated in Gey's balanced salt solution. In order to further characterize the factors involved in the prolactin-stimulating activity (PSA) of serum, decidua was incubated in Gey's buffer alone and in buffer containing either FCS, bovine serum albumin (BSA), or human serum (HS). When buffer was supplemented with varying concentrations of FCS, a dose-related PSA was apparent between 0.1% and 10%. The PSA of FCS was not altered by either heating (85° C for 30 minutes), boiling, or dialysis. The protein fraction of FCS, precipitated with trichloroacetic acid, redissolved in dilute NaOH, and dialyzed against Dulbecco's phosphate buffer, demonstrated PSA comparable to that of whole serum. A similar concentration of BSA added to Gey's buffer did not stimulate the production of PRL by decidua. The PSA of human serum obtained from euprolactinemic men and women was not different from that of FCS. These data indicate that FCS and HS contain a substance(s) (1) which stimulates the secretion of PRL by human decidua, (2) which is very stable, being resistant to dialysis, boiling, and denaturation by trichloroacetic acid, (3) and which is most likely a large polypeptide with a molecular weight greater than 12,000 or a smaller moiety tightly bound to a protein. (AM J. OBSTET. GYNECOL. 138:665, 1980.)

EXPLANTS of human decidua[1, 2] and of chorion with adherent decidua[3, 4] synthesize and secrete immunoreactive prolactin (PRL). When decidua is incubated in oxygenated Gey's buffer, the amount of PRL produced is significantly increased if the salt solution is supplemented with fetal calf serum.[5, 6] In the present study the prolactin-stimulating activity (PSA) of fetal calf serum (FCS) was further investigated in order to partially characterize the factor(s) involved. The effect of human serum (HS) upon the in vitro production of PRL by decidua was also determined and compared to that of FCS.

Material and methods

Fetal membranes were removed from the placenta and washed in normal saline solution immediately after delivery. The amnion was separated from the chorion and discarded. The decidua was dissected from the maternal surface of the chorion, minced with scissors, washed in cold, oxygenated Gey's buffer,[7] and aliquoted by weight (300 mg portions) into 50 ml Erlenmeyer flasks that contained 20 ml of incubation medium. Two flasks were frozen for subsequent analysis in order to determine the initial PRL content of the tissue. The remainder of the flasks were sampled, gassed with 95% oxygen and 5% carbon dioxide, tightly stoppered, and placed in a metabolic shaker maintained at 37° C. These flasks were sampled and regassed during a 24-hour incubation period. At the end of this time, the tissue in each flask was homogenized in the remaining incubation medium, and the supernatant was frozen to be assayed for PRL.

PSA of FCS. In a series of experiments, samples of decidua were incubated in duplicate flasks that contained Gey's buffer or Gey's buffer supplemented with

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Presented at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980.

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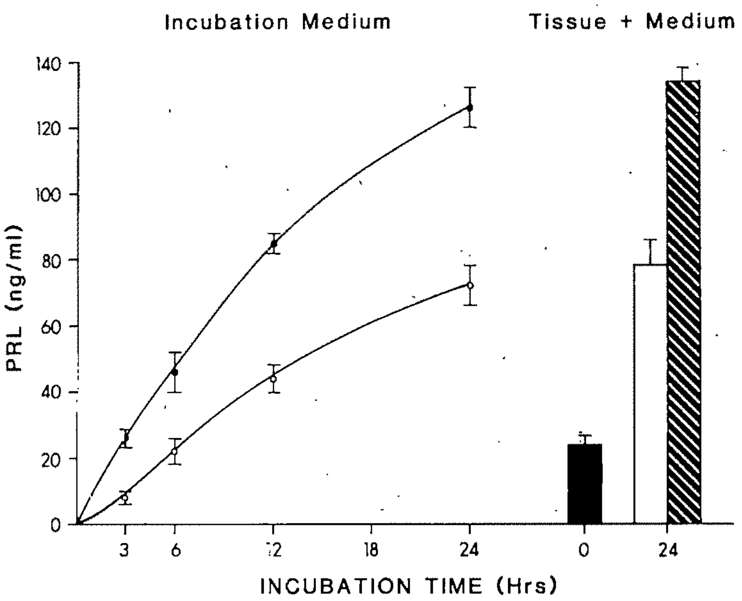

Fig. 1. Production of PRL by human decidua during a 24-hour incubation with (●——●) and without (○——○) fetal calf serum. Each point represents the mean ± SEM of the average PRL values measured in samples of medium from duplicate flasks in a representative experiment. The bars represent the total amount of PRL present initially (solid bar) and after 24 hours' incubation with (hatched) and without (open) FCS in the medium.

0.1%, 1%, 10%, or 20% of FCS (Microbiological Association). The PSA of the FCS was determined by comparing the amount of PRL produced in the enriched medium with that produced in buffer alone. Additional experiments included duplicate flasks in which bovine serum albumin (BSA) (Sigma Chemical Co.) was added to Gey's buffer in order to provide a concentration of nonspecific protein equivalent to the protein content of 10% FCS in Gey's buffer. The PSA of BSA was compared to that of FCS.

In a second series of experiments, FCS was treated as follows: (1) Heat-labile proteins were denatured. FCS was either heated for 30 minutes in a water bath maintained at 85° C or boiled for 5 minutes. (2) Small-molecular weight moieties were removed. Aliquots of 50 ml of FCS were dialyzed at 4° C for 48 hours against 8 L of Dulbecco's phosphate buffer.[8] Cellulose dialysis bags (Thomas Scientific Co.) with a pore diameter of 48 Å were used in order to retain substances with a molecular weight of ≥ 12,000. (3) The acid-precipitable protein fraction was isolated. The proteins present in FCS were precipitated by the addition of 10% trichloroacetic acid (TCA). After centrifugation for 20 minutes at 28,000 × g, the supernatant was removed. The pellet (precipitated protein fraction) was dissolved in 6N NaOH and deionized water to the original volume (50 ml). This solution was then dialyzed for 48 hours against 8 L of phosphate buffer in order to remove the TCA and to restore the pH to 7.2.

Each preparation of treated FCS was tested for PSA in several experiments, each with the use of decidua obtained from a different set of membranes. Decidual tissue was incubated in duplicate or triplicate flasks that contained Gey's buffer or Gey's buffer supplemented with either 10% treated FCS or 10% untreated FCS. PSA was determined by comparing the amount of PRL produced in the presence of treated FCS with that produced in Gey's buffer alone.

PSA of HS. Serum was obtained from euprolactinemic male and female volunteers of reproductive age. In each case the serum PRL concentration was <20 ng/ml, and the final concentration of PRL in Gey's buffer supplemented with 10% HS was <2 ng/ml. The protein fraction of HS was precipitated with saturated ammonium sulfate (SAS) to a final concentration of 50% SAS. After centrifugation for 20 minutes at 28,000 × g, the supernatant was removed, and the pellet (the precipitated protein fraction) was dissolved in deionized water to the original volume of 25 ml. The solution was then dialyzed in 8 L of phosphate buffer for 48 hours to remove the ammonium sulfate ions and restore the original pH of 7.2. The PSA was determined by comparing the amount of PRL produced by the decidual tissue incubated in buffer that contained

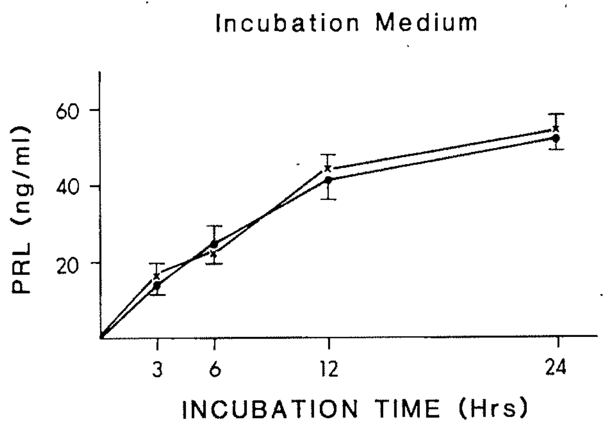

Fig. 2. Accumulation of PRL in Gey's buffer alone (•——•) and in Gey's buffer supplemented with bovine serum albumin (×——×). Each point represents the mean ± SEM of the average PRL values measured in samples of medium from duplicate flasks in a representative experiment.

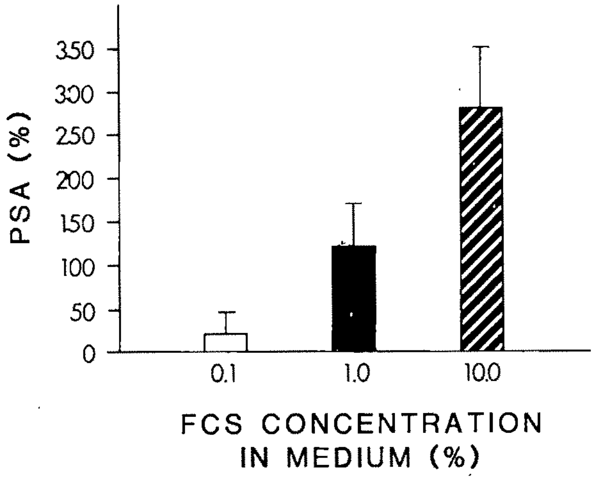

Fig. 3. PSA of different concentrations of FCS present in the medium during a 24-hour incubation. Each bar represents the mean and SEM of eight different experiments.

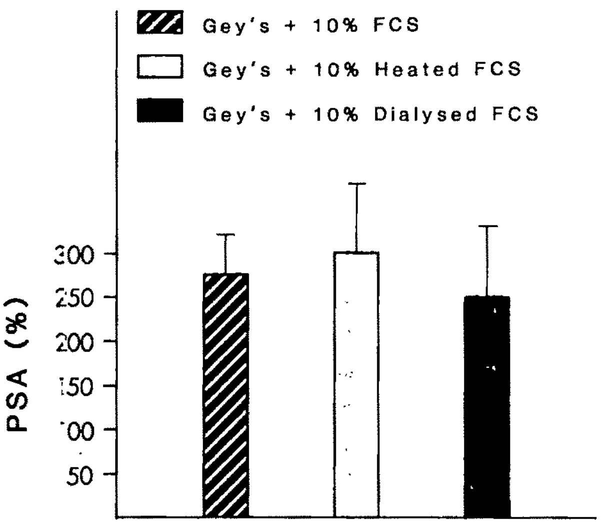

Fig. 4. PSA of FCS heated or dialyzed prior to addition to the incubation medium. The mean ± SEM of four experiments is shown.

either 10% HS or 10% redissolved protein fraction of HS, with that produced in Gey's buffer alone. In these experiments, samples of decidua were also incubated in Gey's buffer supplemented with 10% FCS, so that the PSA of HS could be compared to that of FCS.

PRL and protein assays. Concentrations of PRL in HS, incubation medium, and tissue homogenate supernatants were measured by radioimmunoassay based on the technique of Sinha and associates.[9] Human prolactin (HPr-V-LS2) and rabbit antisera (HPr-V-LS2 and HPr-V-LS3) were provided by the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland. Radioimmunoassay data were analyzed by computer program based on the model developed by Burger and associates.[10] All samples from each experiment were analyzed in duplicate in the same assay; the mean intra-assay coefficient of variation was 5.7%.

Concentrations of protein in the enriched incubation medium (Gey's buffer supplemented with serum or serum proteins) were determined by the method of Lowry and associates.[11]

Statistical analysis. Data were analyzed by the two-tailed Student's t test, with significance defined as $P < 0.05$.

Results

Fig. 1 shows the pattern of accumulation of PRL in the medium during a 24-hour incubation of equal amounts of decidua from the same patient in Gey's buffer alone and in Gey's buffer containing 10% FCS. The medium that contained 10% FCS had significantly higher concentrations of PRL than Gey's buffer alone at all sampling intervals ($P < 0.05$). Concentrations of PRL were also determined in the homogenates of tissue plus medium initially and after 24 hours' incubation. The PRL content of the homogenates after incubation in Gey's buffer was three times greater than that present prior to incubation. Homogenates of decidua incubated in Gey's buffer supplemented with 10% FCS contained nearly six times as much PRL as was present prior to incubation. Thus, not only was the amount of PRL released into the medium increased by the presence of FCS, but the total amount of PRL produced was increased, as well ($P < 0.01$). Since FCS might

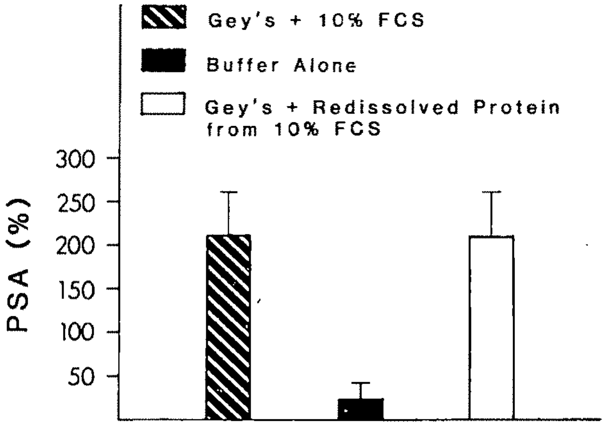

Fig. 5. PSA (mean ± SEM for six experiments) of buffer alone compared to that of buffer containing FCS or the redissolved protein fraction from FCS.

simply provide a nonspecific protein substrate that resulted in an apparent stimulation of PRL synthesis, a similar concentration of albumin was added to Gey's buffer in place of 10% FCS. In this instance, no significant difference in the amount of PRL released during incubation was observed when compared to Gey's buffer alone (Fig. 2).

The effect of increasing concentrations of FCS on total PRL in the tissue plus medium after a 24-hour period of incubation is shown in Fig. 3. Because of the large variability in total secretion of PRL by decidua obtained from different membranes, the data from several experiments (n = 8) are expressed as the percentage increase in PRL produced by the decidua incubated in varying concentrations of FCS compared to that produced in Gey's buffer alone. Minimal PSA was observed when the concentration of FCS in Gey's buffer was 0.1% or less. PSA was present at FCS concentrations of 1% and was increased still further at concentrations of 10% ($P < 0.05$). No additional PSA was observed at concentrations greater than 10%.

To further characterize the chemical nature of the PRL-stimulating factor or factors present in FCS, the effect of heating and dialysis of FCS prior to the addition of it to Gey's buffer was investigated (Fig. 4). Since no difference was noticed in the PSA of heated versus boiled FCS, the data from these experiments were pooled. Neither heating nor dialysis significantly altered the PSA of FCS.

The protein content of FCS ranged between 30 and 40 mg/ml. In order to examine the effect of FCS proteins on PSA, sufficient TCA was added to FCS to effectively precipitate most of the measurable protein. Less than 0.05 mg/ml protein remained in the supernate after this treatment. Fig. 5 shows the PSA of buffer alone, redissolved proteins precipitated from FCS, and untreated FCS when added to Gey's buffer containing equal amounts of decidua and incubated for 24 hours. The PSA of the redissolved protein fraction was not significantly different from that of untreated FCS. Thus, PSA resides in the protein fraction of FCS precipitated by TCA.

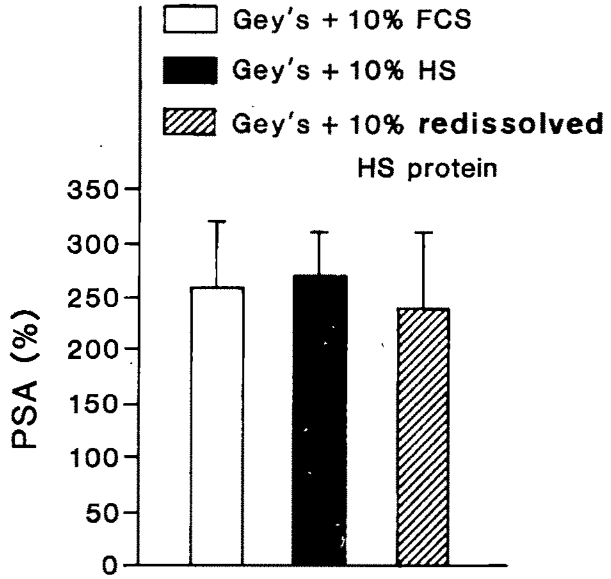

Fig. 6. PSA (mean ± SEM for 3 experiments) of FCS, HS, and redissolved protein fraction from HS.

If the PSA of FCS were of possible physiologic significance, it should also be present in HS, as well. To investigate this possibility, parallel experiments were done in which the decidua was incubated in Gey's buffer alone, Gey's buffer that contained 10% FCS, and Gey's buffer that contained either 10% HS or 10% redissolved proteins precipitated from HS. The results of these experiments are shown in Fig. 6.

Comments

There is now substantial evidence that PRL in amniotic fluid is derived, in large part, from PRL synthesized and released by human decidua. This evidence includes: (1) the lack of correlation between maternal, fetal, and amniotic fluid levels of PRL under both physiologic and pharmacologic conditions[6, 12–15]; (2) a direct and highly significant correlation between the levels of PRL in amniotic fluid and both the content of PRL in the decidual tissue and its ability to synthesize and release PRL in vitro throughout gestation[16]; and (3) the rapid transport of decidual PRL across the chorion in vitro when compared to the transport of radiolabeled PRL added to the medium bathing the fetal membranes.[17]

Virtually nothing is known about the factors that control the synthesis and release of PRL by human decidua, although there is evidence that the control mechanisms may be significantly different from those of the pituitary gland. For example, (1) bromocriptine, dopamine, and thyrotropin-releasing hormone (TRH), potent modulators of pituitary production of PRL, have no effect on the synthesis and release of PRL by human decidua,[18, 19] as well as no effect on PRL in amniotic fluid.[13]

Since FCS is the only substance reported to date which apparently stimulates the production of PRL by decidual tissue, it seemed important to further characterize the substance or substances causing the stimulatory effect. The initial characterization of this factor(s) reported here would suggest that it is either a large-protein moiety or smaller compound tightly bound to a protein. PSA is contained in the TCA-precipitable protein fraction of FCS and is unaffected by heating or dialysis. Woolf and associates[20, 21] have described a factor in HS with physical characteristics similar to those described in this report which increased the production of PRL by dispersed rat pituitary cells in vitro. The factor in HS was twice as potent as FCS in their system, whereas the PSA of FCS and HS on human decidua appeared to be similar. It is possible that this factor(s) could represent a common step in the control of production of PRL by pituitary cells and decidua in vitro, but a more general and unassociated effect is also possible

We wish to express our appreciation to Ms. Sally Kuslis for her technical assistance and to Ms. Darlene Havanec and Ms. Diane Schafer for their help in the preparation of this manuscript.

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Comparison of continuous transcutaneous and continuous intravascular Po_2 measurement in fetal sheep

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Continuously monitored carotid arterial Po_2 (Pa_{O_2}) and transcutaneous Po_2 ($tcPo_2$) from the scalp of fetuses from ewes not in labor have been compared under epidural anesthesia at different levels of fetal oxygenation. A reasonably good correlation existed between the two readings at normal and high Po_2 values. In sharp contrast, at low Pa_{O_2} levels there was a considerable bias between the two. Since this bias was variable, the failure of $tcPo_2$ to reflect Pa_{O_2} accurately at low Pa_{O_2} renders the use of the $tcPo_2$ electrode questionable in the very situation for which it was advocated, namely, the prediction of fetal compromise. (AM. J. OBSTET. GYNECOL. 138:670, 1980.)

INTRAPARTUM fetal asphyxia is probably the major cause of preventable perinatal mortality and morbidity. The prediction and accurate assessment of intrapartum fetal asphyxia would be of considerable practical use in obstetrics. Transcutaneous Po_2 ($tcPo_2$) measurement by means of a thermostatically heated electrode placed upon the fetal scalp has been practiced as a method of intrapartum fetal monitoring.[1] In this study of 51 patients, a correlation was observed between various parameters of fetal heart rate and $tcPo_2$. We previously reported the use of a continuously recording intravascular Po_2 electrode for up to 41 days in the chronically catheterized fetal sheep preparation and demonstrated continuous variability in fetal Po_2.[2] The accuracy with which continuous $tcPo_2$ readings reflect continuous variability in fetal vascular Po_2 has not been determined. Therefore, we compared the values obtained under epidural anesthesia from the continuously recording Po_2 electrode located in the fetal carotid artery (Pa_{O_2}) and those from a transcutaneous electrode applied to the scalp of the fetal sheep. No previous comparison has been reported in the literature for continuously recorded $tcPo_2$ and Pa_{O_2} in the fetus. In addition, the present study was performed without the complication of general anesthesia. The present investigation was conducted in order to provide data that will assist in the correct evaluation of $tcPo_2$ in relation to Pa_{O_2} under different conditions. The findings also throw light on the physiologic changes that occur in cutaneous blood flow in the fetus under differing levels of fetal oxygenation.

Methods

Eight date-mated pregnant Rambouillet-Columbia ewes at 108 to 133 days' gestation were used for this study. Catheters were placed at various points in the fetal circulation and in the amniotic cavity.[2, 3] A continuously recording intravascular Po_2 electrode was placed in the fetal carotid artery.[2] At least 5 days after the first surgical procedure, a second one was performed. The ewes were sedated with an intramuscular dose of 10 mg of 2.6 xylidine dihydrothiazine (Rompun, Bayer). Thirty minutes later, epidural anesthesia was achieved with the administration of 5.0 ml of 2% (w/v) lignocaine (Xylocaine, Astra) and 5.0 ml of 0.5%

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Sponsored by the Society for Gynecologic Investigation.

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0002-9378/80/220670+07$00.70/0 © 1980 The C. V. Mosby Co.

Table I. Fetal and maternal pH and blood gas values*

	At start of premedication	*At start of experiment*	*After hyperoxia*	*After hypoxia*	*At end of experiment*
Fetal pH	7.35 ± 0.05	7.21 ± 0.09	7.17 ± 0.10	7.16 ± 0.08	7.01 ± 0.14
Fetal Pco_2	44.8 ± 5.4	50.8 ± 6.4	51.6 ± 11.1	54.5 ± 10.2	65.2 ± 19.4
Fetal Po_2	19.1 ± 3.6	17.6 ± 3.6	16.3 ± 4.1	15.4 ± 4.0	14.6 ± 4.3
Maternal pH	7.47 ± 0.03	7.49 ± 0.02	7.48 ± 0.02	7.48 ± 0.03	7.48 ± 0.03
Maternal Pco_2	31.6 ± 3.0	33.6 ± 2.9	30.4 ± 5.0	31.1 ± 2.5	37.9 ± 7.4
Maternal Po_2	98.5 ± 6.5	75.8 ± 16.9	65.0 ± 14.4	71.0 ± 8.4	61.2 ± 16.1

*Mean ± SD; n = 3 to 7 as described under "Results," during the experimental protocol. Samples of fetal blood were drawn from the carotid artery, and samples of maternal blood were obtained from the femoral artery at the start of premedication, after stabilization of the Po_2 electrode, after the hyperoxia procedure, after the hypoxia procedure, and at the end of the experiment.

(w/v) bupivacaine (Marcaine, B.D.H.). With the ewe placed in a supine position, the uterus was exposed via a lower midline abdominal incision.

After the maternal abdomen had been opened, the fetal head was located, and in order to make a uterine window of about 5 cm in diameter, an incision was made through the uterus at a position that was free of cotyledons. The occipital area of the fetal head was approximated against the incision to prevent the loss of amniotic fluid. A relatively flat area of the scalp was shaved and a transcutaneous Po_2 electrode, developed by Huch and associates[1] and commercially available from Dräger, West Germany, was fixed to the fetal skin with a cyanoacrylate glue. The technical details of this electrode have been extensively reported elsewhere.[1] The Dräger $tcPo_2$ electrode was chosen because it is reportedly superior to commercially available alternatives.[4] The electrode was heated to 44° C, and the relative heating energy required to produce this stabilization was recorded continuously. At least 30 minutes were allowed to elapse after placement in order to permit stabilization of the $tcPo_2$.

After stabilization of the $tcPo_2$ electrode output, the responses to three experimental protocols were examined in a fixed order: first, with the ewe breathing 100% O_2 (12 L/min) through a transparent plastic bag over her head (hyperoxia studies); second, with the ewe breathing 9% O_2 and 3% CO_2 in N_2 (12 L/min) (hypoxia studies); and, finally, with clamping of the umbilical cord for 1 minute during the administration of 100% O_2 (12 L/min) to the ewe. The period of exposure to maternal hyperoxia was 20 minutes, followed by a period of 20 minutes in which the ewe breathed room air. Ewes were exposed to hyperoxia from two to four times. The protocol for the hypoxia experiments was similar to that for hyperoxia, with the exception that the exposure of the mother to the low Po_2 gas mixture lasted only 15 minutes. Pa_{O_2} values read from the traces as described in Figs. 1 and 2 were pooled in each animal at equivalent times relating to the exposure. The percentage of oxygen in the plastic bag was monitored by means of a Teledyne oxygen controller (Teledyne Analytical Instruments).

At the termination of the hypoxia experiments, the ewes were allowed to breathe room air for 20 minutes. After this 20-minute period, 100% oxygen was administered to the ewe, and the umbilical cord was located through the uterine incision. After a stable period of at least 10 minutes, the umbilical cord was occluded for 60 seconds. After at least 10 minutes, the occlusion was repeated at least twice on each fetus. The total duration of the experiments was between 3 and 5 hours.

The response time of both electrodes was measured in vitro both before and after the experiment. All of the Pa_{O_2} electrodes used had 95% deflection times of 10 to 15 seconds and the transcutaneous (tc) electrodes had a 95% deflection time of 15 to 22 seconds. Samples of fetal blood were taken at frequent intervals to ensure correct reading of the Po_2 electrode.

Data are presented throughout as mean ± SD.

Results

All eight intravascular Po_2 electrodes were functioning at the start of the second surgical procedure. One intravascular electrode ceased recording for unexplained reasons before, and one during, the maternal hyperoxia experiments. Five of the eight transcutaneous electrodes fulfilled the criteria of Huch and associates[1] after application to the fetal scalp, namely, there was an immediate fall from atmospheric Po_2 within 3 to 5 minutes followed by an increase to a plateau within 20 minutes during thermal heating. In one of these five preparations, the intravascular Po_2 electrode stopped working, as described above. Therefore, a direct comparison between both continuous traces was possible in only four fetuses. Three of the eight transcutaneous electrodes failed to function adequately. One reading was zero Po_2 throughout the whole experiment. An-

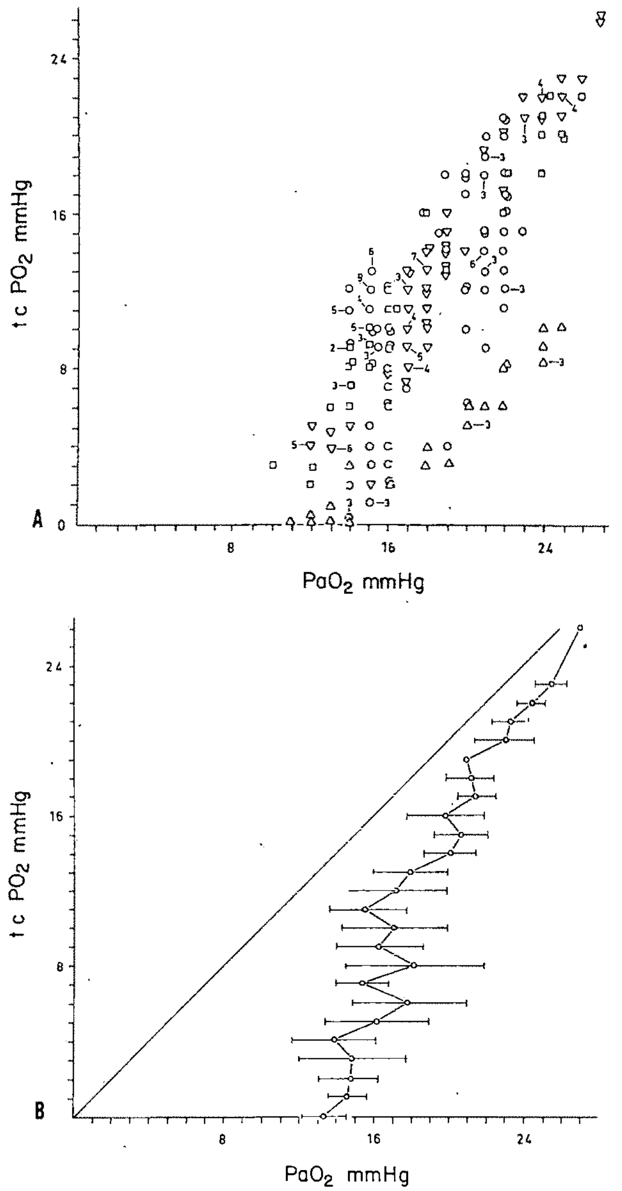

Fig. 1, *A* and *B*. *A*, Comparison of simultaneous measurement of maximum transcutaneous (tc) Po_2 in 2-minute epochs and mean Pa_{O_2} over the same period measured with an indwelling intravascular electrode in four fetal sheep at 114 to 139 days' gestation. Individual symbols represent individual animals, and the numbers represent points at which there was more than one observation. Values obtained during the rapidly changing phase in the first 6 minutes after alteration of the gas mixture inhaled by the ewe and at all times during the cord clamping experiment were not included in Fig. 1. Mean ± SD of Pa_{O_2} values at given $tcPo_2$ values. Data and details as for *A*.

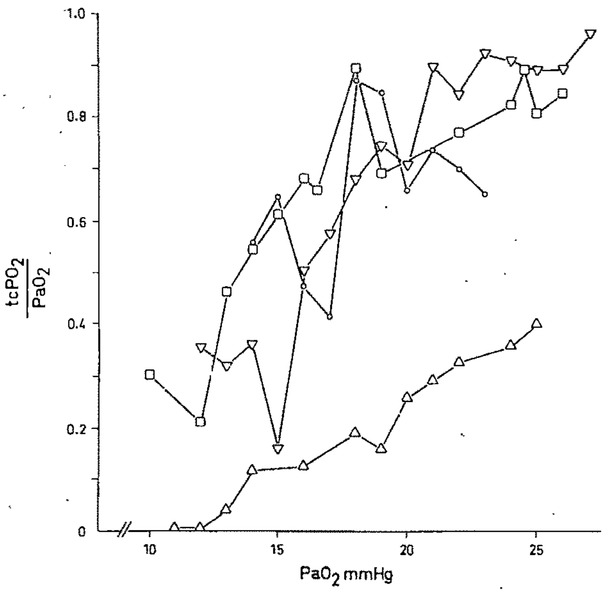

Fig. 1, *C*. Ratio of mean $tcPo_2$ obtained at different Pa_{O_2} values in four fetal sheep at 114 to 139 days' gestation. The total number of data points for the four animals was 79 (△), 35 (□), 25 (▽), and 102 (○) Pa_{O_2} represents the mean of values taken during a 2-minute epoch; the highest $tcPo_2$ during that period was recorded at a given Pa_{O_2}. Mean $tcPo_2$ was calculated from the $tcPo_2$ values measured. Fetus ▽; pH range was 7.34 to 7.26; Fetus □: pH range was 7.13 to 7.10; Fetus ○: pH range was 7.30 to 7.10; Fetus △: pH range was 7.13 to 7.12.

other reading was zero Po_2 except during the hyperoxia experiment, when the reading reached a maximum of 6 mm Hg. A third transcutaneous electrode was rejected since it reached its nadir after 5 minutes of placement. The reading from this electrode rose to only 11 mm Hg $tcPo_2$ when the Pa_{O_2} reached 30 mm Hg during the hyperoxia.

Condition of the fetuses during the experimental protocols. The fetal and maternal pH, Pco_2, and Pa_{O_2} throughout the experiment are shown in Table I. Premedication, epidural anesthesia, and the placement of the ewe in the supine position followed by the surgical procedures described resulted in a fall in fetal pH, although this fall was not statistically significant ($p > 0.05$), and a fall in maternal Po_2, with no marked change in any of the other variables. During the hyperoxia and hypoxia perturbations, there were no marked changes other than a further fall in mean fetal pH of 0.05 mm Hg.

Direct comparison of $tcPo_2$ and Pa_{O_2} in hyperoxic, normoxic, and hypoxic conditions. Fig. 1, *A*, demonstrates the individual gas tensions taken at 2-minute intervals read from the continuous trace as described above. These data were obtained from four fetuses in which a direct comparison between the Pa_{O_2} and tc electrodes was possible. All data points were obtained while the fetus was in a stable condition at least 6 minutes after any change in the composition of the mixtures

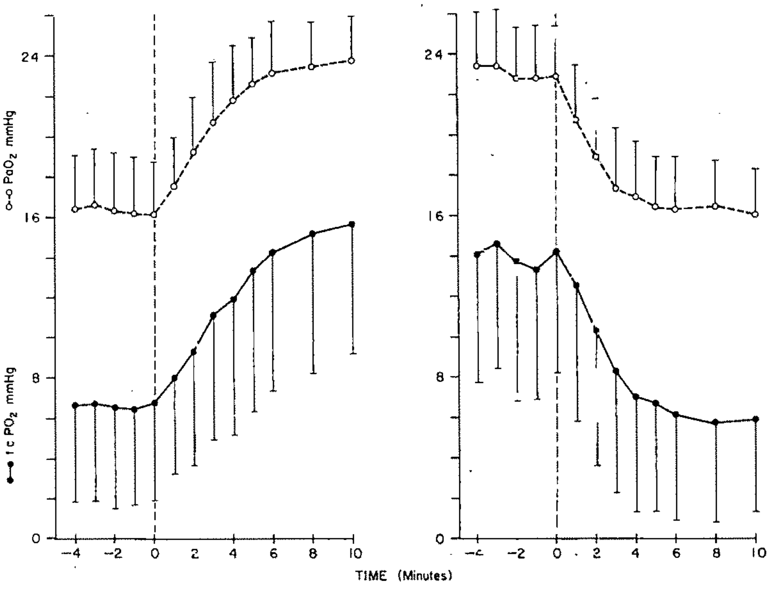

Fig. 2A. Mean ± SD of Pa_{O_2} (o----o) (n = 7) and $tcPo_2$ (•——•) (n = 5) before and during the initial phase of maternal inspiration of 100% oxygen. After a 20-minute period of breathing this gas mixture, the recovery phase is shown.

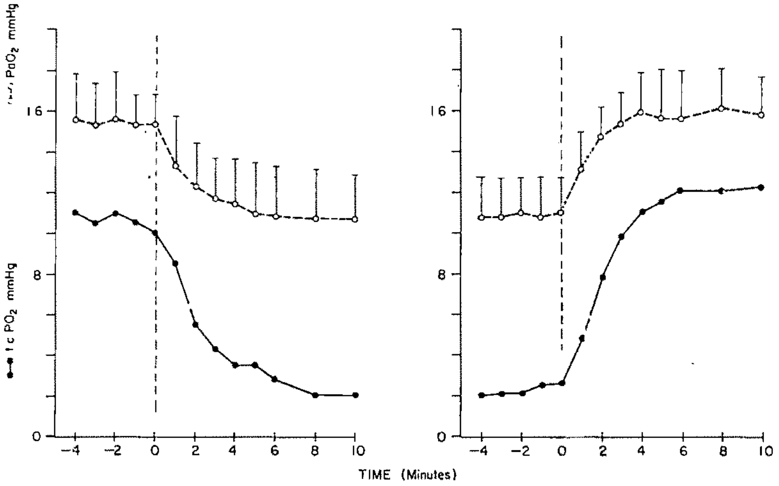

Fig. 2B. Mean ± SD of Pa_{O_2} (o----o) (n = 3) and $tcPo_2$ (•——•) (n = 2) before and during the initial phase of maternal inspiration of 9% oxygen with 3% CO_2. After a 15-minute period of breathing this gas mixture, the recovery phase is shown. Point zero was taken as the moment when the new equilibrium in the bag was reached.

inhaled by the ewe. Po_2 values obtained during equilibrium in response to different gas mixtures are discussed separately below. No data from the cord clamping experiments are included. Fig. 1, *A* and *B*, demonstrates clearly that the divergence of $tcPo_2$ from Pa_{O_2} increases as Pa_{O_2} falls. This observation is also well demonstrated when the ratio of $tcPo_2$ is plotted against Pa_{O_2} (Fig. 1, *C*). In all four fetuses this ratio was always less than 1, even during maternal hyperoxia, and fell as fetal Pa_{O_2} decreased. In one fetus the ratio was always less than 0.5, which was considerably less than that in the other three fetuses. The low value in this animal did not seem to be due solely to the low pH, since another fetus also had the same pH. As the experiments progressed, there was a tendency for the overall ratio of $tcPo_2$ to Pa_{O_2} to fall. In a comparable situation, the mean ratio in the first hyperoxia experiment and the hyperoxia immediately prior to the cord clamping

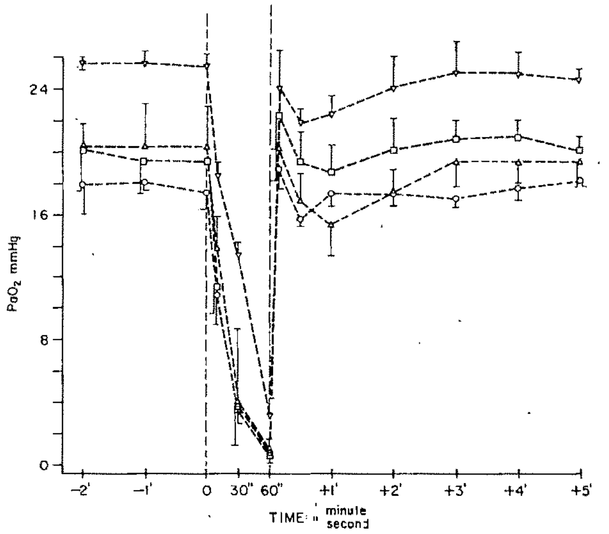

Fig. 3A. Response of fetal Pa_{O_2}, monitored continuously to 1 minute of cord clamping in four fetal sheep at 114 to 139 days' gestation. Each point represents the mean ± SD of three to five episodes.

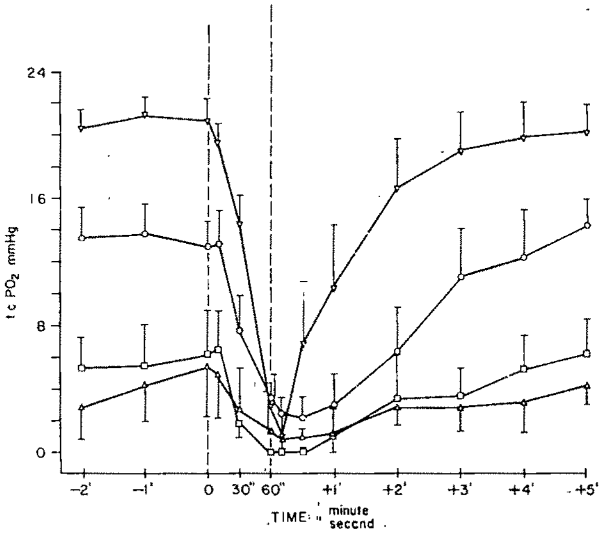

Fig. 3B. Response of fetal $tcPo_2$ to 1 minute of cord clamping (as in Fig. 3*A*).

in the four animals was: 0.92 and 0.84 (▽), 0.42 and 0.20 (△), 0.86 and 0.78 (o), and 0.88 and 0.32 (□).

Response to maternal inhalation of different gas mixtures. The response during maternal hyperoxia was observed in all seven preparations in which the Pa_{O_2} electrodes were working and in all five in which the $tcPo_2$ electrodes were functioning (Fig. 2A). The exposure of the ewe to hypoxia was performed in the two preparations with functional $tcPo_2$ electrodes in which $tcPo_2$ was above 9 mm Hg, and in one fetus in which the Pa_{O_2} electrode alone was working (Fig. 2B).

The increases in Pa_{O_2} and $tcPo_2$ during maternal hyperoxia were similar. The maximum increases in Pa_{O_2} and $tcPo_2$ were 1.64 ± 0.65 mm Hg min^{-1} (n = 7) and 1.78 ± 0.70 mm Hg min^{-1} (n = 5), respectively. The maximum declines from the plateaus when the ewe inhaled room air were 2.23 ± 0.45 mm Hg min^{-1} (n = 7) for the Pa_{O_2} electrode and 2.1 ± 0.65 mm Hg min^{-1} (n = 5) for the $tcPo_2$ electrode.

The maximum changes during maternal hypoxia were a fall of 2 ± 0.87 mm Hg min^{-1} (n = 3) for Pa_{O_2} and 3.0 (mean of 2) mm Hg min^{-1} for the $tcPo_2$ electrode. When the ewe breathed room air, the Pa_{O_2} electrode reading rose maximally, 2.1 ± 0.2 mm Hg min^{-1} (n = 3), and the $tcPo_2$ rose 3.0 mm Hg min^{-1} (mean of 2).

Response of $tcPO_2$ to clamping the umbilical cord for 1 minute. Figs. 3A and 3B illustrate the response of four fetuses to clamping the umbilical cord for 1 minute. The response of Pa_{O_2} was statistically significant at 10 seconds ($p < 0.05$). The fall in $tcPo_2$ occurred more slowly, and in none of the fetuses did the $tcPo_2$ fall significantly within the first 10 seconds.

The differences in the recovery period were even more marked. Pa_{O_2} returned to normal within 10 seconds, whereas recovery of $tcPo_2$ to its initial levels required up to 5 minutes.

Relative heating power. The relative energy required to keep the temperature of the electrodes stable at a plateau of 44° C, which is said to be an indicator of the state of vasodilation in the skin below the electrode, did not reveal any recognizable pattern during any part of the experimental protocol.

Comment

Since the $tcPo_2$ electrode has been advocated for the at-risk fetus, despite the lack of experimental data, in order to assess its clinical usefulness, we investigated the effects of hyperoxia and hypoxia on the correlation between the Pa_{O_2} and $tcPo_2$. The fetal pH and Pa_{O_2} values that existed after the procedures which were required for the placement of the transcutaneous electrode were similar to those observed in many at-risk human fetuses.

To our knowledge, no previous comparison has been made of these two continuously recording methods of monitoring fetal oxygenation. In one study in which a comparison was made between $tcPo_2$ and samples of blood obtained intermittently from the fetal carotid artery, a good correlation was observed over the range of 14 to 28 Hg.[5] In this report, neither the timing of the samples nor the period of exposure to different gas mixtures (approximately 5 minutes) was specified. In one figure, $tcPo_2$ was shown to fall to nearly zero; however, no values lower than 14 mm Hg were used in the correlation between Pa_{O_2} and $tcPo_2$.

In another study, in two fetuses under general anesthesia with Ethrane, the same comparison of intermittent Pa_{O_2} and continuous $tcPo_2$ yielded an $r = 0.977$ and a slope of 0.89.[6] Only two of the 18 points used for this calculation were at a Pa_{O_2} of less than 14 mm Hg. The remainder ranged from 14 to 34 mm Hg. Ethrane is a halogenated hydrocarbon, and anesthetics of this group, including halothane, have been shown to be polarographically reduced at Po_2 electrodes[7-9] and are best avoided when one is making measurements of Po_2. In addition, general anesthesia of the fetus may affect the level of peripheral vasoconstriction, thereby modifying any disparity between $tcPo_2$ and Pa_{O_2} measurements wherever this might otherwise exist. All forms of anesthesia, general and regional, may modify fetal oxygenation and regional blood flow.

In one study of newborn monkeys sedated with Sernylan (phencyclidine HCl), $tcPo_2$ and Pa_{O_2} correlated well at a Pa_{O_2} of 70 mm Hg and above, but when Pa_{O_2} was lowered to 25 mm Hg, $tcPo_2$ fell to zero.[10] These results in the newborn animal show a similarity to the disparity that we have demonstrated at low Po_2 in the fetus.

The changes in fetal Pa_{O_2} that we have observed during and after maternal hyperoxia agree partly with observations published previously by Matalon and associates.[11] They observed a slightly different rate of increase in Pa_{O_2}, 2.44 mm Hg min^{-1} versus our finding of 1.64 mm Hg $min,^{-1}$ and decrease, 1.63 mm min^{-1} versus our finding of 2.23 mm $min.^{-1}$ However, the conditions of their experiment differed considerably; in particular, the ewes were under general anesthesia with tracheal intubation and were mechanically ventilated.

It is of interest that Pa_{O_2} readings from the intravascular electrode at 1 minute after occlusion of the umbilical cord were lower than those previously reported in the literature.[12] The Pa_{O_2} values obtained from the indwelling intravascular Pa_{O_2} electrode were almost certainly superior to those obtained from samples of blood measured extracorporeally, for two reasons. First, in our experience, accurate assessment of very low Po_2 values necessitates specific calibration in the low range. This observation is in accordance with other reports in the literature, in which a blood gas analyzer in 100% N_2 gas flow read 11 ± 1.4 mm Hg Po_2.[13] Second, for practical reasons, during intermittent sampling under experimental conditions in an animal research laboratory, it is extremely difficult to obtain blood under the absolutely anaerobic conditions necessary to measure low blood Po_2 accurately. The zero Po_2 leakage current was always determined in our electrodes both before the insertion and after the experiment, and the reading was corrected for this value.

Our data subsequent to occlusion of the umbilical cord bear resemblance to published data on the delayed response of the transcutaneous Po_2 after restoration of fetal blood flow.[14] In this study, fetal oxygenation was impeded by occlusion of the maternal aorta, and after a 2-minute period of occlusion the transcutaneous Po_2 required about 10 minutes to reach its original value. However, the recorded fall in $tcPo_2$ was to 6 mm Hg, whereas the intra-arterial Po_2, calculated from the oxygen saturation, fell to nearly zero. The $tcPo_2$ value of 6 mm Hg can be explained solely by the halothane effect, whereas the oxygen saturation will not be influenced by halothane.

The divergence of Pa_{O_2} at low Po_2 during hypoxia and the cord clamping experiments was most likely due to vasoconstriction in the skin. Pronounced peripheral vasoconstriction in the carcass in response to hypoxemia, accompanied by similar levels of acidemia (pH 7.28), has been observed.[15] Peripheral vasoconstriction in response to hypoxemia would enable blood to be redistributed to more vital organs.

We were unable to associate relative heating power with any changes in Pa_{O_2}. Changes in the relative heating power have been suggested as an indicator of fetal peripheral vasoconstriction, but it yielded no useful information in our study. We have observed both in the experimental and in the clinical obstetric situation that the relative heating power changed when the electrode was touched during vaginal examination. The close correlation demonstrated between the relative heating power and uterine contractions during labor in the human being may well be artifactual and not reflect physiologic changes in relative local perfusion.

Our experiments were designed to eliminate extraneous physical factors, such as the so-called tonsure effect of the cervix,[16] the presence of a caput succedaneum, and pressure upon the electrode. It is difficult to assess the contribution that these factors might make to $tcPo_2$ measurements in the human fetus during labor, but pressures as low as 30 to 113 grams (equivalent to 8 to 13 mm Hg) upon the electrode placed upon the scalp of two adults reduced the reading to zero.[17] However, since the avascular layer in the adult skin is much thicker, $tcPo_2$ readings in this situation are much lower than the intra-arterial Po_2. In this study, intra-arterial Po_2 was not measured. If we assume a value of 100 mm Hg for Pa_{O_2}, the initial ratio of $tcPo_2$ to Pa_{O_2} was only 0.14 and 0.38, so that extrapolation from adults to neonates or fetuses is probably inappropriate.

In conclusion, we have demonstrated a pronounced and unpredictable bias between $tcPo_2$ and Pa_{O_2} at low fetal Pa_{O_2} levels. Clinically, the $tcPo_2$ electrode has been advocated for use in the at-risk fetus. Our data strongly question the value of this electrode in the hypoxic fetus.

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Effect of adrenergic agonists on progesterone production by human placental cells in culture

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Human placental cells maintained in culture were used to determine the direct effect of beta-adrenergic agonists on progesterone accumulation by the placenta. In five studies, when either of the beta-adrenergic receptor agonists, terbutaline or isoproterenol, 1.5 μg/ml, was incubated for 24 hours with the placental cells, significantly more progesterone was present in the culture medium than in the medium of matched controls. Compared with the medium of matched controls, progesterone in medium increased 36 ± 6% (mean ± SE) when cells were treated with terbutaline and 38 ± 7% when cells were treated with isoproterenol. In two dose-response studies with terbutaline, a minimal dose of 0.15 μg/ml was required to significantly increase progesterone in medium compared with that in matched controls. In three studies, propranolol at a concentration of 1.5 μg/ml completely blocked the stimulatory effect of 1.5 μg/ml of terbutaline. (AM. J. OBSTET. GYNECOL. 138:677, 1980.)

BETA-ADRENERGIC agonists effectively inhibit preterm labor.[1] Recent studies in animals have indicated that these agents stimulate the production of progesterone by bovine[2] and ovine[3] luteal cells in vitro; therefore, it is possible that an analogous effect may be observed in the human placenta. Clinical studies have provided conflicting data in this regard. Csapo and Herczeg[4] noted that beta-adrenergic agonists increased progesterone in the peripheral plasma of pregnant women, but Bibby and associates[5] reported a decrease in the peripheral progesterone concentration of pregnant women who were receiving these agents. In both of these studies, measurements were made during labor, a time when the concentration of progesterone in peripheral blood may be altered by the effect of the beta-adrenergic agonist on labor and uterine blood flow. In the present study, placental cells maintained in culture were used to determine the effect of beta-adrenergic agonists on progesterone production by the human placenta, thereby eliminating the uterine effects of these agents as a variable.

Material and methods

Placentas were obtained aseptically from women who were undergoing elective repeat cesarean section. The placentas were processed within 1 hour by modification of the technique described by Hall and associates.[6] Several fragments of fetal cotyledons, approximately 5 by 5 by 3 cm each, were dissected from the placenta and were washed with 0.15M NaCl to remove excess blood. These fragments were finely minced with a scalpel until a homogeneous suspension of villi was obtained. Fifty milliliters of trypsin (0.25% w/v, Worthington Corporation, Freeport, New Jersey) was added to approximately 15 grams of the minced tissue, and this mixture was incubated at 37° C for 10 minutes in an oscillating water bath. The supernatant containing dispersed placental cells was removed, and 1 ml of fetal calf serum was added. The supernatant was then centrifuged at 225 × g for 10 minutes. The pellet was resuspended in 5 ml of Weymouth's MB752/1 culture medium (GIBCO, Grande Island, New York) supplemented with 10% fetal bovine serum (v/v). Occasionally during trypsin digestion a mucoid suspension would form, trapping the harvested cells. In such cases, 200 units of deoxyribonuclease (Worthington Corporation, Freeport, New Jersey) dissolved in 1 ml of 0.15M NaCl was added. The minced tissue was treated with trypsin five

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Presented at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 20, 1980.

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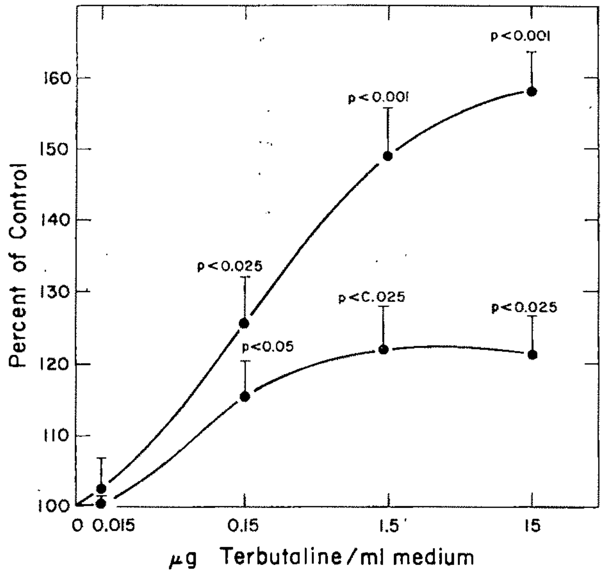

Fig. 1. Dose-response for terbutaline in two placentas. Each value represents mean (±SE) of six wells. Each value is compared to its control.

Table I. Progesterone in medium (ng progesterone/well/24 hours) after 24-hour incubation of placental cells with and without adrenergic stimulation

Placenta	*Control*	*Terbutaline (1.5 μg/ml)*	*Isoproterenol (1.5 μg/ml)*
1	25 ± 2	35 ± 2†	30 ± 1*
2	53 ± 2	87 ± 7†	78 ± 3†
3	16 ± 1	19 ± 1†	23 ± 1†
4	34 ± 2	44 ± 2†	51 ± 4†
5	38 ± 3	49 ± 5	48 ± 6

Values are mean (±SE) of six wells.

*$p < 0.025$, compared with matched control by analysis of variance and Newman-Keuls multiple range test.

†$P < 0.01$, compared with matched control by analysis of variance and Newman-Keuls multiple range test.

to six times. Harvested cells from each treatment were pooled, and equal aliquots of cells were dispensed into culture dishes (Costar, Cambridge, Massachusetts) containing six wells (35 by 15 mm) each. Three to six culture dishes were used per placenta, depending on the type of study. Cell counts were approximately 2 to 5 × 10^5 per well, depending on the number of culture dishes used per placenta. An exact cell count could not be obtained because the cell preparation consisted primarily of aggregates of cells whose boundaries could not be readily delineated. The culture dishes were maintained in a humidified atmosphere of 5% CO_2 for 3 hours at 37° C until the placental cells had attached. The culture medium which contained unattached placental cells, red cells, and other debris was aspirated, and the attached placental cells were washed with 1 ml of fresh culture medium. Dishes were then selected at random either to serve as controls or to receive treatment with a study drug. Medium alone or medium containing terbutaline,* a specific β_2 adrenergic receptor agonist (Astra Pharmaceuticals, Worcester, Massachusetts), isoproterenol,† an adrenergic agonist with β_1 and β_2 receptor activity[7] (Breon Labs, New York, New York), or propranolol, a beta-adrenergic antagonist (Ayerst Labs, New York, New York), was added to each well, and the dishes were returned to the CO_2 incubator. Each dish of six wells received the same treatment. After 24 hours of incubation, the medium was removed and centrifuged at 225 × g for 10 minutes, and the supernatant was frozen until analyzed for progesterone by specific radioimmunoassay.[8] The sensitivity of the assay was 50 pg/ml. Reactivity with 17-OH progesterone and 20α-dihydroprogesterone was less than 1% of that for progesterone.[8] Ten placentas were studied. Differences in the accumulation of progesterone between culture dishes were compared by analysis of variance and the Newman-Keuls multiple range test.[9] Although data were expressed as a percent of control in some figures, statistical analyses of the data were always performed with the data expressed as nanograms progesterone per well.

Results

The cells in culture were similar morphologically to those described by Thiede.[10] The predominant cell was epithelial, which had abundant cytoplasm and a large pale nucleus with prominent nucleoli. These epithelial cells commonly aggregated to form a syncytial-like mass with many nuclei. A second type of epithelial cell with a large, dark-staining nucleus and scant cytoplasm was infrequently observed. Long fibroblast-like cells were also present, and the percentage of these cells varied in individual experiments. Viability of attached placental cells determined by exclusion of 0.1% trypan blue was greater than 95% 24 hours after initial plating.

In five placentas, the effect of terbutaline and isoproterenol on the accumulation of progesterone in the culture medium was compared with matched controls (Table I). In the five experiments, terbutaline, 1.5 μg/ml (6.6 × 10^{-6}M), increased progesterone in the medium by 36 ± 6% (mean ± SE) compared with matched controls. In four of these studies, the increase in progesterone was significantly greater than in matched controls, but in the fifth study the increase did not reach statistical significance ($p = 0.1$). Similarly, isoproterenol, 1.5 µg/ml (7.0×10^{-6}M) increased progesterone in medium by 38 ± 7% (mean ± SE) compared with matched controls, and the increase was statistically significant in all but one experiment.

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*Terbutaline sulfate dissolved in physiologic saline solution.

†Isoproterenol hydrochloride with lactic acid, Na lactate, NaCl, and Na bisulfite.

In five additional placentas, the response of cultured placental cells to beta-adrenergic agonists was further characterized. Two dose-response studies with terbutaline were performed, and in three studies an attempt was made to block the stimulatory effect of terbutaline on the accumulation of progesterone by the specific beta-adrenergic antagonist, propranolol.

Fig. 1 demonstrates the results of the two dose-response studies. In both experiments, terbutaline at a concentration of either 0.15, 1.5, or 15 µg/ml of medium significantly increased the accumulation of progesterone in medium, compared with matched controls. In one experiment, the response to terbutaline reached a plateau at a dose of 1.5 µg/ml. In the second experiment, the response to terbutaline did not plateau with a dose of 15 µg/ml. For the two placentas, the accumulation of progesterone was 20% greater than control with a dose of 0.15 µg terbutaline/ml, 32% greater than control with a dose of 1.5 µg/ml, and 40% greater than control with a dose of 15 µg/ml.

The results of one of the three experiments with propranolol blockade are depicted in Fig. 2. Similar results were obtained in the two other placentas studied. Terbutaline, 1.5 µg/ml, produced a significant ($p < 0.001$) increase in progesterone accumulation compared with matched controls. The addition of 0.15 µg/ml of propranolol (6.0×10^{-7}M) reduced the stimulatory effect of terbutaline on the accumulation of progesterone, and propranolol, 1.5 µg/ml (6.0×10^{-6}M), completely prevented the stimulatory effect of terbutaline. Propranolol alone had no effect on the accumulation of progesterone when compared with controls.

Comment

The present study indicates that the addition of either beta-adrenergic agonist, terbutaline or isoproterenol, to placental cells maintained in culture significantly increases the accumulation of progesterone in culture medium when compared with matched controls. The response to terbutaline is dose related and is blocked by the beta-adrenergic antagonist, propranolol.

Increased accumulation of progesterone after incubation of placental cells with adrenergic agonists may be due to either decreased metabolism, increased biosynthesis, or increased release of progesterone. Our data do not indicate which of these processes account for the stimulatory effect observed here. However, Winkel and associates[11] reported minimal metabolism of radiolabeled progesterone by placental cells maintained in culture under conditions similar to those of our experiments, thus suggesting that altered progesterone metabolism may not be responsible for our findings. On the other hand, beta-adrenergic agonists may act directly on placental cells to increase the production and/or release of progesterone. Condon and Black[2] and Jordan and associates[3] have shown that beta-adrenergic agonists increase the production of progesterone in bovine and ovine corpus luteum cells, and that this response occurs in parallel with an elevation in intracellular cyclic adenosine monophosphate (AMP). It is well established that cyclic AMP modulates steroidogenesis in a variety of steroid-producing tissues.[12] A similar effect of beta-adrenergic agonists on cyclic AMP and steroidogenesis may be operative in the human placenta. Indeed, Whitsett and associates[13] have demonstrated beta-adrenergic receptors and catecholamine-sensitive adenyl cyclase activity in membrane fractions of human placentae, thereby supporting the earlier studies of Satoh and Ryan.[14] Furthermore, either exogenous cyclic AMP or its dibutyl derivative augments estrogen biosynthesis[15] and human chorionic gonadotropin release[16] by placental cells in vitro. Therefore, it is possible that the increased progesterone accumulation we observed after the incubation of placental cells with beta-adrenergic agonists may have been due to a specific response of the placental cells to the agonists, and may have been mediated by an increase in intracellular cyclic AMP.

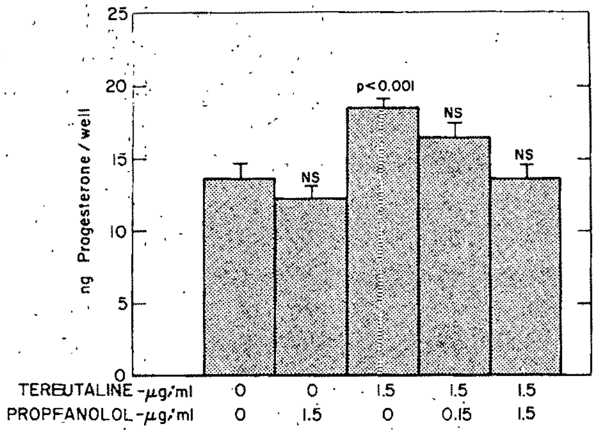

Fig. 2. Effect of propranolol blockade on terbutaline-stimulated progesterone accumulation in medium. Height of bar represents mean (±SE) of six wells. Dose of drugs is indicated under each bar. Each treatment is compared with the matched control.

The concentration of adrenergic agonist which increased progesterone accumulation in vitro may be achieved clinically during the inhibition of labor. In vitro, we saw a significant increase in progesterone accumulation with a concentration of terbutaline of 0.15 μg/ml. The concentration of terbutaline during intravenous therapy for preterm labor has not been reported, but a single intravenous injection of 250 μg will result in a peak serum concentration of 0.025 μg/ml in nonpregnant subjects.[17] Because pregnant women receive up to 15 times this amount hourly for the treatment of preterm labor,[18] the serum concentration achieved may be similar to that which causes increased accumulation of a progesterone in vitro.

Although our data indicate that beta-adrenergic agonists increase the accumulation of progesterone by placental cells maintained in culture, it remains to be proved whether these agents will affect the production of progesterone by the placenta in vivo.

We wish to thank Karen King for her diligence in performing the radioimmunoassay for progesterone, and Harold Schuler for staining the placental cells.

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Decidual production of prolactin in late gestation: Further evidence for a decidual source of amniotic fluid prolactin

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The capacity of human decidual tissue to synthesize prolactin de novo throughout late gestation was investigated and correlated with the levels of prolactin (PRL) in amniotic fluid. Maximal concentrations of PRL in both amniotic fluid and samples of decidua were found prior to the thirtieth week of gestation and declined simultaneously until term. A high correlation ($r = 0.90$, $p < 0.00005$) was found when the levels of PRL in amniotic fluid and the initial (preincubation) content of PRL in decidua from the same patient were compared. A very high correlation ($r = 0.96$, $p < 0.00005$) was seen between the ability of the decidua to produce prolactin in vitro and the corresponding levels of prolactin in amniotic fluid. No significant difference in any parameter tested was noted with respect to either the sex of the fetus or the mode of delivery ($p > 0.05$). These data are interpreted as indicating (1) that decidual tissue varies throughout late gestation, in both its initial content of prolactin and its ability to synthesize prolactin de novo, in a manner which correlates to a high degree with variations in amniotic fluid prolactin levels and (2) that the decidual tissue is the major source of amniotic fluid prolactin. (AM. J. OBSTET. GYNECOL. 138:681, 1980.)

ENDOMETRIAL stromal tissue transformed into decidua has been demonstrated to synthesize prolactin (PRL) whether its origin is parietal decidua in normal gestation,[1–3] decidua in endometrial samples taken during the late luteal phase of the normal menstrual cycle,[4] or decidua obtained from endometrial curettings after the removal of tubal ectopic pregnancies.[5] This prolactin has been shown to be indistinguishable from human pituitary prolactin, by the criteria of immunoprecipitation and gel chromatography,[1, 6, 7] and electrophoretic and receptor assay techniques.[7]

Since large amounts of PRL are found in human amniotic fluid throughout gestation,[8–12] much speculation as to the source of the PRL has been engendered. Maternal and/or fetal pituitary glands were considered by some to be potential sources of amniotic fluid prolactin, but several observations tend to refute these suggestions.[9, 13, 15–17]

The present study was undertaken to investigate the capacity of human decidua to produce prolactin throughout late gestation and to determine whether this capacity correlates with the levels of PRL in amniotic fluid.

Methods

Decidual tissue was obtained at delivery from fetal membranes in 88 pregnancies after either spontaneous labor or cesarean section between 26 and 42 weeks' gestation. Wherever possible, amniotic fluid was also obtained either at the time of, or just prior to, delivery. The fetal membranes were cut free of the placental disc and the amnion was removed and discarded. The chorion with adherent decidua was then washed three times in cold normal saline solution, incubated for 20 to 30 minutes at room temperature in normal saline solu-

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This work was supported in part by the National Foundation–March of Dimes.

Presented in part at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980.

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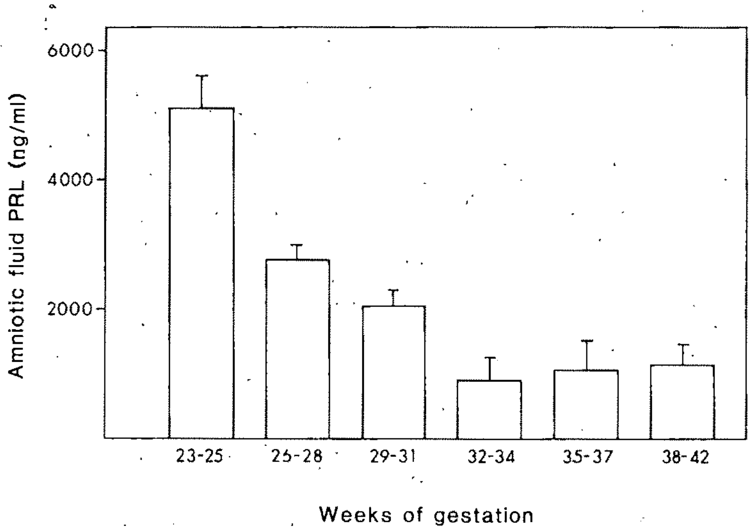

Fig. 1. Amniotic fluid prolactin levels in late gestation.

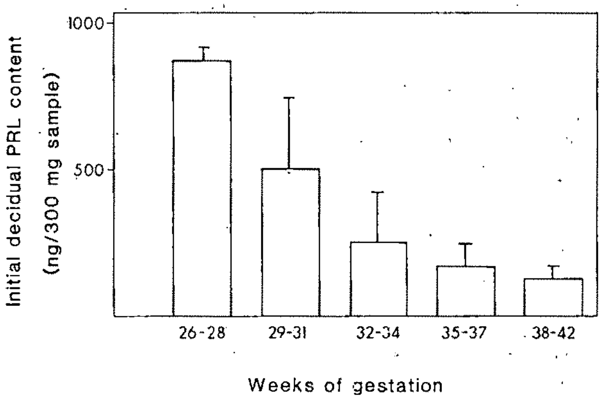

Fig. 2. Preincubation content of prolactin in decidual tissue in late gestation.

tion (containing 1 mM of EDTA), and rinsed in saline solution a final time. The decidua was dissected from the chorion, placed in cold oxygenated Gey's buffer, and minced with small scissors. This tissue was then washed three times in cold oxygenated Gey's buffer and divided into aliquots of 300 mg (±3%), wet weight, and placed in Ehrlenmeyer flasks. Twenty milliliters of oxygenated Gey's Buffer supplemented with 5% fetal calf serum and penicillin (2,500 IU), streptomycin (2,500 μg), and gentamicin (100 μg) were added to each flask. Two flasks were frozen for subsequent analysis. The remaining flasks were then sampled, regassed with 95% O_2–5% CO_2, tightly stoppered, and incubated 24 hours at 37° C in a Dubnoff metabolic shaker. At the end of the incubation, the tissue in each flask was homogenized in the remaining buffer, and the supernatant was assayed for prolactin.

Prolactin assay

Prolactin concentrations in all samples and tissue homogenates were estimated by radioimmunoassay based on the technique of Sinha and associates.[18] Human prolactin (HPrV-LS2) and rabbit antisera (AFP #2) were provided by the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland. Radioimmunoassay data were analyzed by computer program based on the model developed by Burger and associates.[19] Samples of medium and/or amniotic fluid which contained high concentrations of prolactin showed slopes parallel to the pituitary prolactin standard over a 50-fold dilution range. Intraassay and interassay variabilities were less than 5%.

Statistical analyses were performed by means of Scheffe's test for multiple comparisons, and linear regression analysis where indicated. (All data were tested by logarithmic, power, and exponential transformations before linear analysis was accepted as appropriate.)

Results

Amniotic fluid PRL levels throughout late gestation in this series of patients are shown in Fig. 1. Higher concentrations were observed in the more premature patients. Significant differences ($p < 0.01$) occurred between groups until approximately 32 weeks' gestation, and there was no significant difference beyond that point. These data are similar to the findings reported by Chochinov and associates[12] and others.[8-11]

The initial content of PRL in decidua (i.e., prior to incubation) followed a very similar pattern (as seen in

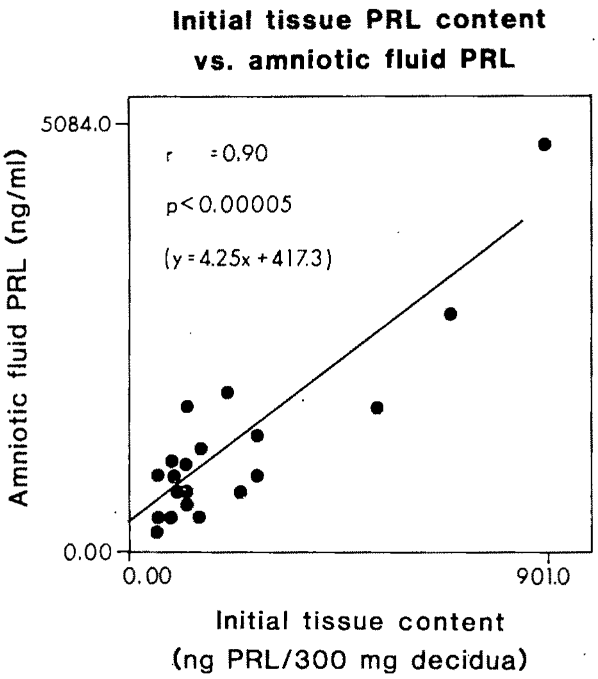

Fig. 3. Correlation of preincubation content of prolactin in decidual tissue and prolactin in amniotic fluid in the same patients.

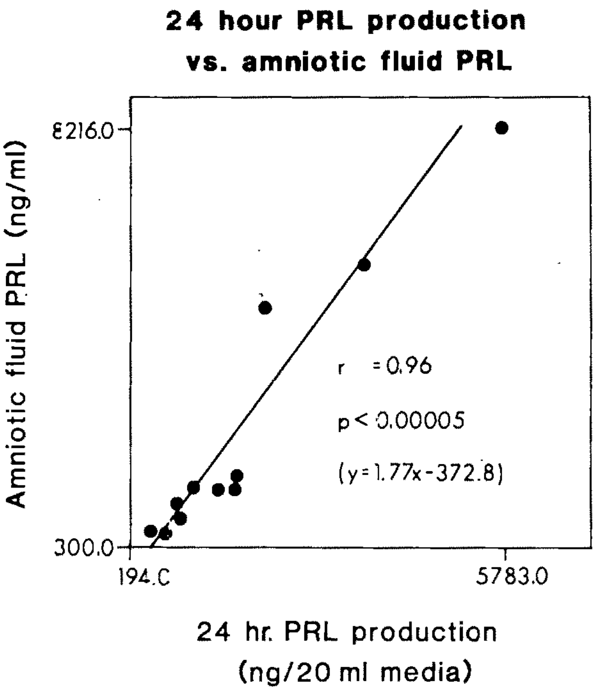

Fig. 4. Correlation of 24-hour decidual production of prolactin in vitro and amniotic fluid prolactin in the same patients.

Fig. 2), with the highest hormone levels noted near the end of the second trimester. Again, from 32 weeks' gestation on, no significant differences were found.

To test the hypothesis that amniotic fluid PRL levels are indeed related to initial decidual content of PRL in the same patient, a linear regression with the use of the data from Figs. 1 and 2 is shown in Fig. 3. The correlation was 0.9 ($p < 0.00005$). A similar analysis was constructed in order to compare amniotic fluid PRL levels with the amount of PRL produced by samples of decidua in vitro. Fig. 4 shows the correlation of 24-hour production of PRL by the decidual specimen and the amniotic fluid PRL in the same patient. That the decidual capacity to produce PRL is directly related to amniotic fluid PRL is indicated by the correlation coefficient of 0.96, and $p < 0.00005$. Finally, Fig. 5 demonstrates not only that amniotic fluid PRL levels can be predicted both by initial decidual content of PRL and by the capacity of that decidua to produce PRL in vitro, but also that the production capacity can be predicted from the initial tissue content ($r = 0.92$, $p < 0.00005$).

No significant differences in any parameter tested were observed with respect to the sex of the fetus ($p > 0.05$) or the mode of delivery, i.e., vaginal versus cesarean section ($p > 0.05$). (Cesarean section data were not analyzed with respect to the onset of spontaneous labor.)

Comment

Several observations tend to refute the possibility that the major source of amniotic fluid PRL is either the maternal or fetal pituitary gland, or both: (1) suppression of both maternal and fetal pituitary PRL production to very low levels with bromocriptine does not influence amniotic fluid PRL levels[13, 15]; (2) progressive changes in maternal and fetal serum PRL throughout gestation are discordant with changes in amniotic fluid PRL[3]; (3) amniotic fluid PRL levels do not change after maternal hypophysectomy or after intrauterine death of the fetus in rhesus monkeys[16]; and (4) isotopically labeled PRL injected into the pregnant rhesus monkey or fetus shows an extremely low level of transfer to the amniotic fluid.[9, 17] This has led some authors to suggest an extrapituitary source for amniotic fluid PRL.[20] Neither placenta nor isolated fetal membranes with decidua removed are capable of synthesizing PRL de novo, and in 1977, the suggestion was first made that this source was indeed decidua.[21] Since then, others have supported this view.[3, 7, 22]

The present study presents the strongest evidence to date that maternal decidual tissue is the major source of the PRL found in the amniotic compartment. A very high correlation ($r = 0.96$) was found between the concentration of PRL in the amniotic fluid prior to delivery and the ability of the decidua from the same patient to

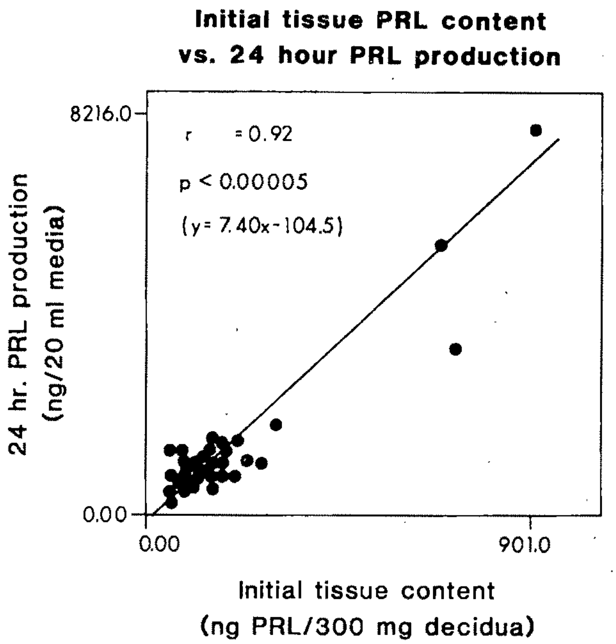

Fig. 5. Correlation of preincubation content of prolactin in decidual tissue and the ability of the same tissue to produce prolactin in vitro.

synthesize PRL in vitro. A similar correlation (r = 0.90) was found between amniotic fluid PRL levels and the initial PRL content of the decidua, prior to incubation.

Thus, the relatively high levels of PRL in amniotic fluid, characteristic of the late second trimester, were associated with decidua which not only contained high levels of PRL prior to incubation, but could also synthesize high levels of PRL in vitro as well. As gestational age increased to term, both the amount of PRL in the amniotic fluid and that in the decidual tissue declined, as did the ability of the decidua to produce PRL in vitro.

These data indicate that the amount of PRL produced in vitro reflects the in vivo condition of the decidua. Thus, explants of tissue obtained after premature delivery (prior to 32 weeks) are capable of significantly higher rates of hormone synthesis during incubation than are tissues obtained nearer to term. The physiologic correlate of this observation is the progressive decline in the amount of PRL present in both the amniotic fluid and in the decidua itself until approximately 32 weeks' gestation. These data strongly suggest that amniotic fluid levels of PRL are dependent on the capacity of decidua to produce PRL de novo.

This view has recently been supported by the observations made by Riddick and Maslar[23] of the transport of prolactin across human fetal membranes. These investigators observed that approximately 40% of the prolactin present in the decidual tissue adherent to intact fetal membranes appeared on the amniotic side of the membranes after incubation, whereas <5% of radiolabeled PRL added to the medium bathing the decidual surface of the membranes subsequently appeared on the amniotic side during a similar period of incubation. A similarly low rate of transfer of radiolabeled PRL occurred across isolated chorion and amnion.

In summary, these data are interpreted to indicate that decidual tissue varies throughout late gestation in both its initial content of prolactin and its ability to synthesize prolactin in a manner which correlates to a very high degree with variations in amniotic fluid prolactin levels, indicating that the decidual tissue is most likely the major source of amniotic fluid prolactin.

We wish to thank Mr. James Yopp, Jr., and Ms. Sally Kuslis for their invaluable technical assistance and Ms. Darlene Havanec for her greatly appreciated help in the preparation of the manuscript.

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Biorhythms of the primate uterus *(Macaca mulatta)* during labor and delivery

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The association between the documented spontaneous biorhythms of uterine activity, uterine artery blood flow, and aortic blood pressure and the processes of labor and delivery was explored in 17 nonmedicated rhesus monkeys for 18 pregnancies. The characteristic circadian variations of higher hourly average values of uterine activity during periods of light than during periods of darkness were incorporated in a statistically distinct, predictable, and repetitive manner into the events of parturition. Variations in uterine artery blood flow reflected changes in intra-amniotic pressure. In those animals in which delivery occurred at night, a shift in maximal values of uterine activity from periods of light to periods of darkness occurred 48 to 60 hours prior to delivery. Progressive linear increases in hourly average values of frequency of contraction and aortic blood pressure consistently began 24 to 36 hours prior to delivery. These changes suggest that specific, directly related prodromal events occur within the maternal organism hours before parturition in the nonhuman primate. (AM. J. OBSTET. GYNECOL. 138:686, 1980.)

SPECIFIC biorhythms in the dynamics of the nonhuman primate uterus have been demonstrated during the last half of pregnancy.[1] Spontaneous uterine activity, as measured by calculation of the average pressure under the contractile curve and by frequency of contraction, follows a time course that is repetitive, predictable, and varies from hour to hour in diurnal patterns that conform to highly significant Fourier curves synchronized by the light-dark cycle. In general, uterine artery blood flow varies inversely to the average values of intra-amniotic pressure. The flow rates are highest during the hours of darkness when intra-amniotic pressure is lowest, and decrease as average pressure values approach their highest level or apogee during the period of light. In late gestation, the difference between the maximal and minimal average values of uterine artery blood flow during the day may be as much as 63% of mean flow. This variation may be modified by the circadian changes in aortic blood pressure.

The present study was undertaken to characterize the patterns of uterine activity and hemodynamics during parturition and to investigate the relationship of the biorhythms in spontaneous uterine dynamics observed during late pregnancy to the processes of labor and delivery.

Method

Intra-amniotic pressure, frequency of uterine contraction, aortic blood pressure, and uterine artery blood flow were measured chronically in nonmedicated rhesus monkeys conditioned to restraining chairs. The pattern of changes in amniotic fluid pressure was recorded by transabdominal, intra-amniotic insertion of an open-end, fluid-filled catheter connected by way of a pressure transducer to a recording polygraph. Values of aortic blood pressure were recorded from an open-end, fluid-filled catheter threaded through the femoral artery to a level between the bifurcation of the aorta and the renal arteries. Direct measurement of uterine artery blood flow was achieved by means of an electromagnetic flow meter. The flow probe was placed on the uterine artery, contralateral to the blood pressure

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Supported in part by Research Grant HD-02798 from the National Institutes of Health, United States Public Health Service.

Sponsored by the Society for Gynecologic Investigation.

Preliminary findings presented at the Twenty-fourth Annual Meeting of the Society for Gynecologic Investigation, Tucson, Arizona, March 23-25, 1977.

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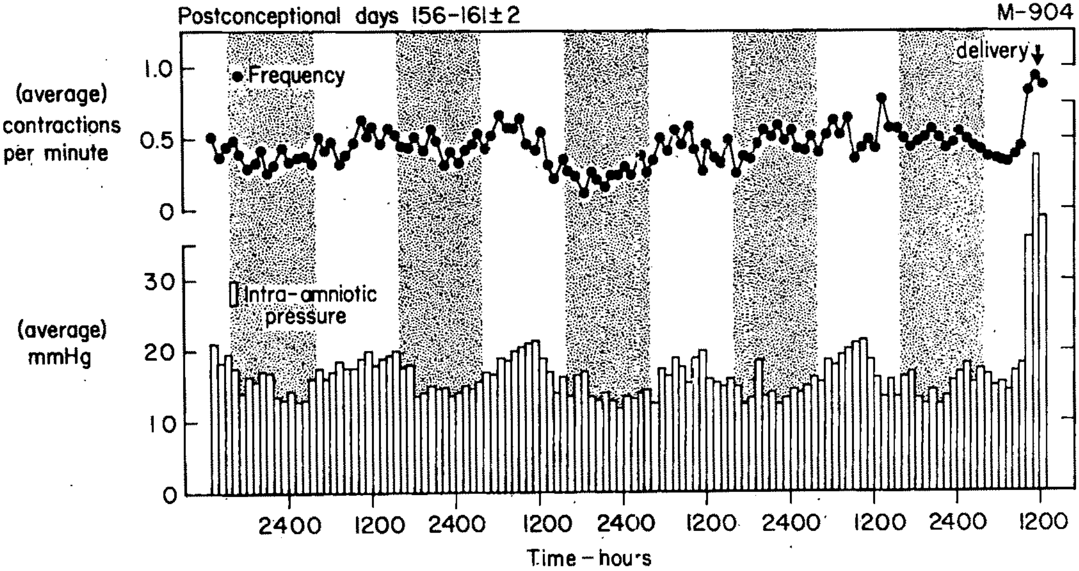

Fig. 1. Illustration of the variations in uterine activity recorded over 5 consecutive days during late pregnancy, labor, and delivery. Data bars and points represent the average values of intra-amniotic pressure and frequency of contraction for each of the 120 hours of continuous monitoring that culminated in delivery during a period of light. The alternating 12-hour cycles of light and dark are represented by the clear and stippled background areas, respectively.

catheter. Placement, calibration, and maintenance of the sensors were accomplished by previously established techniques.[1]

As soon as the operation was completed, the animal was placed in a restraining chair and allowed to recover from anesthesia in the recumbent position. Three to 5 days were allowed for seating of the flow probe before "chronic" measurements were undertaken. The animals were housed in a controlled environment and maintained on alternating 12-hour cycles of dark and constant-intensity light. All experimental variables were monitored continuously, with the animal maintained in an upright, sitting posture throughout the onset of spontaneous labor and delivery.

To facilitate quantitative evaluation of the changes occurring in the patterns of the pressure and flow signals, mean pressure and flow were determined electronically with the use of a 1-second time constant. These sensor signals were fed on-line into integrating preamplifiers calibrated so that the writing styluses traveled 50 mm in 30 seconds when receiving a signal equal to one half of the measurement scales. Total activity, the area under the polygraph curve, was calculated by multiplying the number of millimeters traveled by the stylus by the appropriate factor. Average values of mean pressure and flow were calculated by dividing the total integrated activity by the specified time interval in seconds. Frequency of contraction was expressed as the average number of complete contraction-relaxation complexes occurring above baseline pressure per minute.

Results

The onset of spontaneous labor and delivery were studied for 18 pregnancies in 17 monkeys. The time interval from operation until delivery ranged from 8 to 24 days. Satisfactory continuous measurements of uterine artery blood flow in chronic preparations were obtained in 10 animals, and of aortic blood pressure in 11. Postconceptional duration of pregnancy was known within ±2 days, and on the date of delivery varied from 144 to 165 days. In nine monkeys, delivery occurred during the 12-hour period of light, and in nine it occurred during the period of darkness.

One animal was followed through two successive pregnancies that occurred 13 months apart. The hourly average values of uterine activity recorded during the 5 days terminating in delivery at 1246 hours, during the period of light, are displayed graphically in Fig. 1. The biorhythms of uterine activity observed during late pregnancy[1] are reiterated, with the highest hourly average values of intra-amniotic pressure and frequency of contraction occurring during the periods of light or early evening, and the lowest values during the periods of darkness or early morning. The high hourly average values of intra-amniotic pressure re-

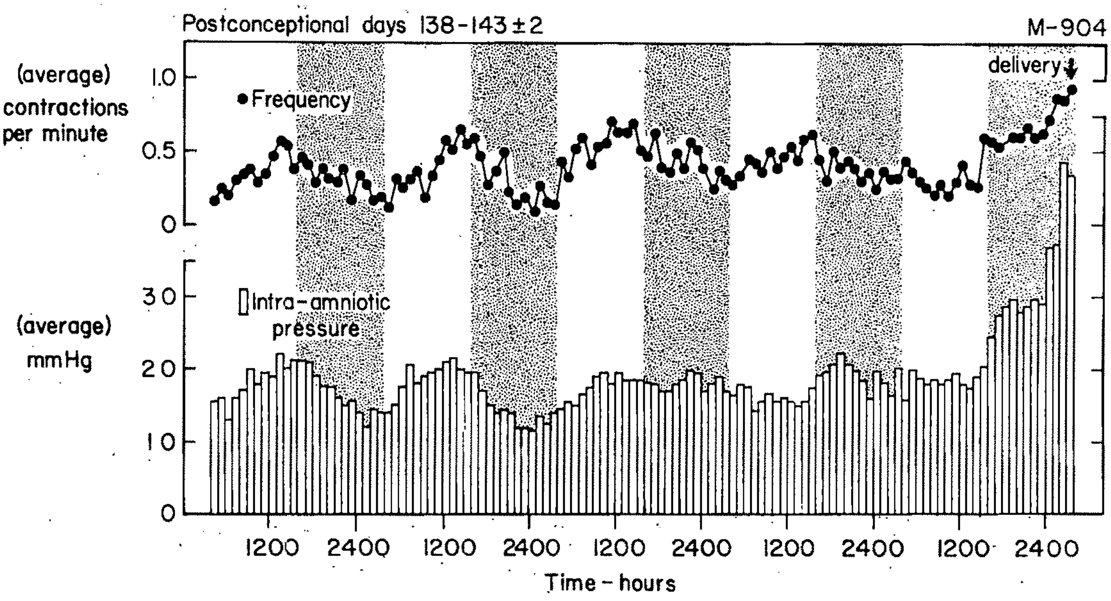

Fig. 2. Hourly average values of uterine activity recorded during a subsequent pregnancy in the same animal as in Fig. 1. Delivery occurred during the period of darkness. A shift in the relationship of maximal values of intra-amniotic pressure and frequency of contraction to the periods of light and dark occurred 48 to 60 hours prior to delivery.

corded approximately 24 to 30 hours before delivery occurred during the period of evolution from the irregular, low-amplitude contractions of late pregnancy to the simple, coordinated, contractile pattern characteristic of prelabor.[2] A decrease in average intra-amniotic pressure and frequency of contraction was observed in the 6- to 8-hour period prior to delivery and before the onset of contraction complexes characteristic of active labor. The time from onset of these uniform contractions until delivery was 3 hours and 16 minutes.

Fig. 2 displays the hourly average values of uterine activity recorded from 120 hours of continuous monitoring during the subsequent pregnancy in Monkey 904, when delivery occurred at 0357 hours, during the period of darkness. Despite the differences between light and dark and in gestational age at the time of delivery, a sinusoidal pattern of average intra-amniotic pressure values, with a decrease preceding the progression into the higher values of labor and delivery, was noticed again. However, on the day prior to delivery, the lowest average values of intra-amniotic pressure occurred during the period of light, whereas the highest values were observed during the period of darkness. This shift in circadian rhythm occurred 48 to 60 hours prior to delivery and immediately followed a prolonged period of increased uterine activity that extended from the higher hourly average values recorded during the period of light on the preceding day. Frequency of contraction displayed a similar phase shift. In this pregnancy, the duration of active labor, evidenced by high-amplitude, uniform contractions, was 7 hours and 43 minutes. For the other 16 deliveries, the length of active labor ranged from approximately 50 minutes to almost 9 hours.

The analysis of variance for Fourier terms[3] indicated that the spontaneous variations in hour-to-hour values of average intra-amniotic pressure resulting in delivery could be characterized by statistically significant Fourier curves ($F_{2,21} = 22.81$ for Fig. 1, and 14.72 for Fig. 2). However, for both deliveries, the multiple correlation coefficients (0.634 and 0.583) indicated that there was considerable scatter of the actual values about the calculated curves. The changes in hourly average values of contraction frequency in Fig. 1 did not conform to a statistically significant Fourier pattern ($F_{2,21} = 1.68$). The changes in frequency in Fig. 2 conformed to the equation $y = 0.20 + 0.03 (\cos ct) - 0.15 (\sin ct)$, where ct represented time in radians ($F_{2,21} = 73.10$; multiple correlation coefficient 0.950).

In an effort to better characterize the observed changes mathematically, we subjected to analyses of variance for regression[3] the hourly average values of intra-amniotic pressure and frequency of contraction. Comparisons made for Fourier curves with component exponential, linear, logarithmic, or power functions calculated from values recorded in another monkey over a 36-hour period terminating in delivery during

Table I. Analysis of variance for regression of hourly average values of uterine activity recorded over a 36-hour period terminating in delivery on postconceptional day 162 ± 2 (M-201)

		Intra-amniotic pressure (average), mm Hg			*Frequency of contraction (average), contractions/minute*		
Source	*Degrees of freedom*	*Mean square*	*F ratio*	*Multiple correlation coefficient*	*Mean square*	*F ratio*	*Multiple correlation coefficient*
Regression equation: $y = a_0 + a_1 (\cos ct) + b_1 (\sin ct) + c_1 e^{(ct)}$							
Regression on b_1	3	344.25	126.49	0.992	0.4210	5.61	0.344
Residual	32	2.72			0.0750		
Regression equation: $y = a_0 + a_1 (\cos ct) + b_1 (\sin ct) + c_1 (ct)$							
Regression on b_1	3	220.61	15.41	0.591	0.8713	53.31	0.860
Residual	32	14.31			0.0163		
Regression equation: $y = a_0 \div a_1 (\cos ct) + b_1 (\sin ct) + c_1 (\ln ct)$							
Regression on b_1	3	156.84	7.73	0.420	0.5380	8.39	0.440
Residual	32	20.29			0.0640		
Regression equation: $y = a_0 + a_1 (\cos ct) + b_1 (\sin ct) + c_1 (ct)^2$							
Regression on b_1	3	643.72	33.14	0.792	0.6215	11.05	0.509
Residual	32	19.41			0.0562		
Total (uncorrected)	36	443.79			0.2474		
Correction for mean	1	14856.80			5.2441		

ct = Time in radians.

the period of light at 1158 hours are summarized in Table I. The highest F ratio (126.49) and multiple correlation coefficient (0.992), with use of the pressure data, were obtained for the regression equation $y = 18.4 - 0.3 (\cos ct) + 1.8 (\sin ct) + 0.0021 e^{(ct)}$. The variations in hourly average values of frequency of contraction were best described by a regression equation containing both a Fourier and a linear component [$y = 0.51 - 0.13 (\cos ct) + 0.07 (\sin ct) + 0.0747 (ct)$; $F_{3,32} = 53.31$; multiple correlation coefficient = 0.860].

Fig. 3, *A*, presents the hourly average values for 76 hours and the calculated best-fit curve for the last 36 hours of the variations in intra-amniotic pressure and frequency of contraction recorded from a third monkey. Delivery in this animal occurred during the period of darkness. The component curves and the specific equations determined by the analysis of variance for regression are shown in Fig. 3, *B*. The F ratios were highly significant ($F_{3,32} = 97.88$ for pressure and 80.30 for frequency). The multiple correlation coefficients for pressure and frequency were 0.912 and 0.871, respectively. The specific time of highest value or acrophase of the Fourier component of both the pressure and the frequency curves occurred during the early period of darkness, several hours before delivery. The period of change from minimal to progressively increasing values (the transition curve of the exponential function) of changes in amniotic fluid pressure coincided with the ascending portion of the Fourier component. The linear component of the curve best describing the changes in frequency of contractions began more than 24 hours before delivery but well after the shift of the biorhythms associated with higher hourly average values and delivery during the periods of darkness.

Fig. 4, *A*, depicts 76 hourly average values and curves of best fit calculated by the analysis of variance for regression for the last 36 hours of intra-amniotic pressure, aortic blood pressure, and uterine artery blood flow recorded during late pregnancy, labor, and delivery from a monkey in which delivery occurred during the period of light. There was a progressive increase in hourly average values of aortic blood pressure that started 24 to 36 hours prior to delivery. This increase was associated with a gradual elevation in both systolic and diastolic blood pressures during the periods of uterine relaxation, as well as the episodic increases engendered by greater uterine activity. Hourly average values of uterine artery blood flow decreased in a fashion which produced a pattern that was essentially a mirror image of the changes in amniotic fluid pressure. As labor progressed, the hourly average values of uterine artery blood flow successively decreased to levels as low as 58% of the mean flow rate recorded during the 76 hours illustrated (29.9 ml per minute ± 4.3).

The specific regression equations and the component curves for each variable are illustrated in Fig. 4, *B*. The F ratio and multiple correlation coefficient calculated for the composite curve for intra-amniotic pressure were 116.49 and 0.903, respectively. The highest F ratio (97.46) and multiple correlation coefficient (0.869) for the changes in aortic blood pressure were obtained for the regression equation having both a Fourier and a linear component. The curve of best fit

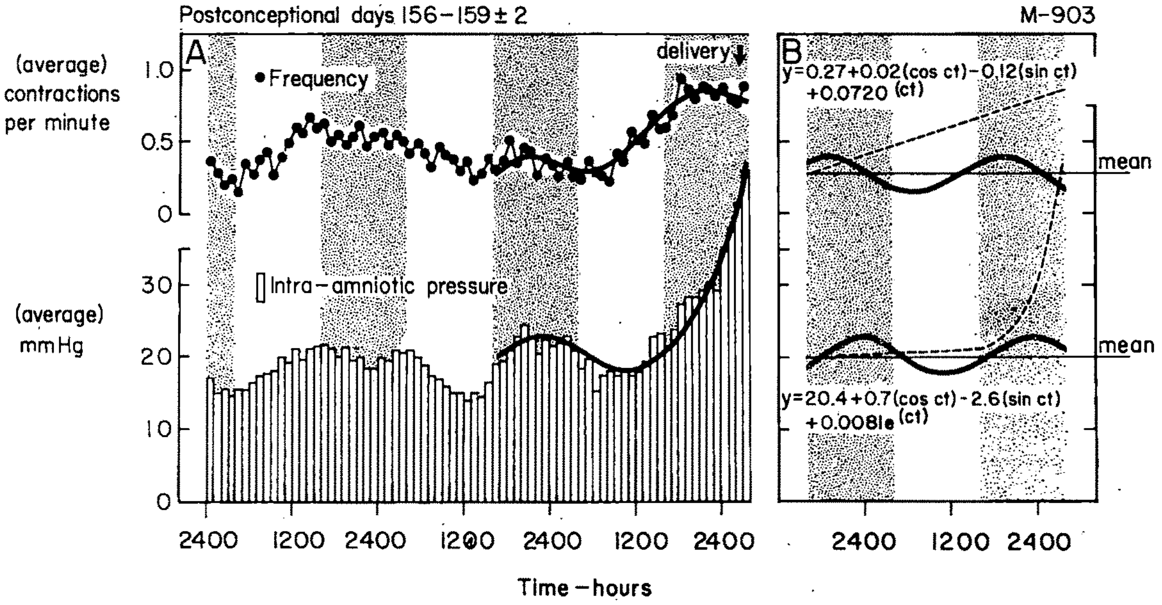

Fig. 3. *A,* Graphic presentation of the hourly average values of intra-amniotic pressure and frequency of contraction recorded over a 76-hour period in a monkey in which delivery occurred at 0346 hours. The two continuous solid lines represent the curve of best fit for pressure and for frequency calculated by the analysis of variance for regression for the last 36 hours of monitoring. *B,* Illustration of the component curves and specific equations of both pressure and frequency changes. The factor *ct* represents time in radians. The highest point or acrophase of the Fourier components, represented by the solid lines, occurred at 2000 hours for pressure and 1924 hours for frequency. The broken lines depict the third component curves for pressure, 0.0081 $e^{(ct)}$ and frequency, 0.0720 (ct).

for uterine artery blood flow was composed of a highly significant sine curve with a peak or semiamplitude of 4.5 ml/min above the mean and a negative exponential function (F ratio 94.26, multiple correlation coefficient 0.844). The transition curve of the exponential component occurred during a descending phase of the Fourier element and after the reciprocal changes in the exponential portion of the intra-amniotic pressure variations. Resistance to uterine blood flow, calculated as the quotient of hourly average values of aortic blood pressure less intra-amniotic pressure divided by corresponding hourly average values of uterine artery blood flow, ranged from 2.57 to 5.97 mm Hg · ml^{-1} · min and was significantly correlated with changes in uterine pressure ($r = 0.927$, $p < 0.001$). The hourly average variations in total uterine vascular resistance were best described by the equation, $y = 3.79 - 0.46 (\cos ct) + 0.22 (\sin ct) + 0.0002 e^{(ct)}$ (F ratio 83.80, multiple correlation coefficient 0.966).

Changes in average values of amniotic fluid pressure, aortic blood pressure, and uterine artery blood flow recorded over a 5-day period in another monkey in which delivery occurred during the period of darkness are presented in Fig. 5. Forty-eight to 60 hours prior to delivery, a shift occurred in the biorhythm of maximal and minimal hourly average values of intra-amniotic pressure in relation to periods of light and dark. Uterine artery blood flow displayed a corresponding shift, with higher hourly average values occurring during the periods of light, when intra-amniotic pressures were lower. However, higher hourly average values of aortic blood pressure continued to occur during the periods of light, and lower values, during the periods of darkness. With the exception of these changes in light and dark relationships, the patterns of change in uterine activity and hemodynamics associated with labor and delivery were similar to those observed when delivery occurred during the period of light. There was a progressive increase in hourly average values of aortic blood pressure that started 24 to 30 hours prior to delivery and reached values 20% above the mean for the 5-day period, 115.4 mm Hg ± 10.5. As labor progressed, the hourly average values of uterine artery blood flow decreased to levels as low as 65% of the mean flow rate recorded during the 120 hours illustrated (32.1 ml/min ± 3.0). Changes in uterine vascular resistance ranged from 2.63 to 4.16 mm Hg · ml^{-1} · min and were closely related to changes in amniotic fluid pressure ($r = 0.921$, $p < 0.001$).

Table II summarizes the data obtained from 12

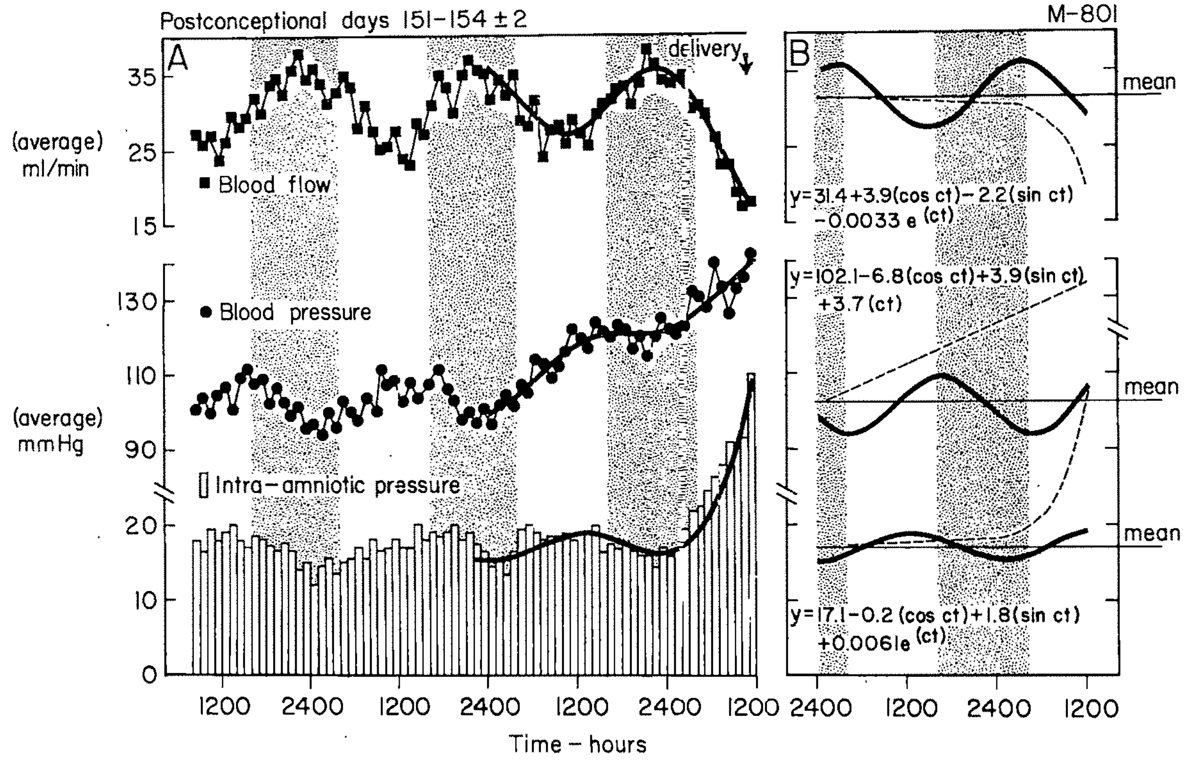

Fig. 4. Graphic presentation of the variations in hourly average values of intra-amniotic pressure, aortic blood pressure, and uterine artery blood flow monitored over 76 hours *(A)*, and the component curves and multiple regression equations calculated for the last 36 hours of monitoring *(B)* in an animal in which delivery took place at 1252 hours. Aortic blood pressure began to increase in a progressive, linear fashion 24 to 36 hours before delivery. The variations in hourly average values of uterine artery blood flow essentially mirrored the changes in intra-amniotic pressure. The hour-to-hour changes in amniotic fluid pressure were linearly related to the variations in both blood flow ($r = -0.835$) and blood pressure ($r = 0.690$). The correlation between blood flow and blood pressure was also statistically significant but inversely related ($r = -0.593$, $p < 0.05$).

other deliveries that were subjected to the analyses of variance for regression. In 11 animals, the curve of best fit to the spontaneous changes in amniotic fluid pressure was comprised of a highly significant sine curve and an exponential element. The F ratios ranged from 63.51 (M-610) to 115.19 (M-908); multiple correlation coefficients ranged from 76.43 (M-010) to 139.35 (M-1909). The highest point or acrophase of the Fourier curve always occurred within 7 hours of the time of delivery and was within 1 hour in five pregnancies. In each instance, the transition phase of the exponential component occurred during the ascending limb of the Fourier curve. The other monkey (M-1905) had a breech presentation and a prolonged labor. The equation $y = 16.4 + 4.7$ (cos ct) + 1.2 (sin ct) + 0.08 (ct) yielded the highest F ratio (53.31) and multiple correlation coefficient (0.605) for the pressure values. The changes in hourly average values of contraction frequency increased in a linear progression during the 24 to 36 hours before delivery for all 12 animals. In three deliveries, a statistically significant Fourier component in the changes of frequency of contraction could not be demonstrated.

The curve of best fit describing the variations in uterine artery blood flow was consistently composed of both a cosine and a negative exponential element beginning after the corresponding changes observed in intra-amniotic pressure values. Even in the animal in which delivery was by breech presentation, the regression equation $y = 26.3 + 3.1$ (cos ct) − 1.8 (sin ct) − 0.0007 $e^{(ct)}$ best described the blood flow variations ($F_{3,32} = 77.29$, multiple correlation coefficient = 0.791). The average blood flow rate during the hour of delivery was 31.3% below the mean flow calculated for the multiple regression equation, 29.7 ml per minute ± 5.2. In all nine animals in which chronic continuous measurements of aortic blood pressures were obtained, the variations in hourly average values were best described statistically by the regression equation, $y = a_0 + a_1$ (cos ct) + b_1 (sin ct) + c_1 (ct). The F ratios

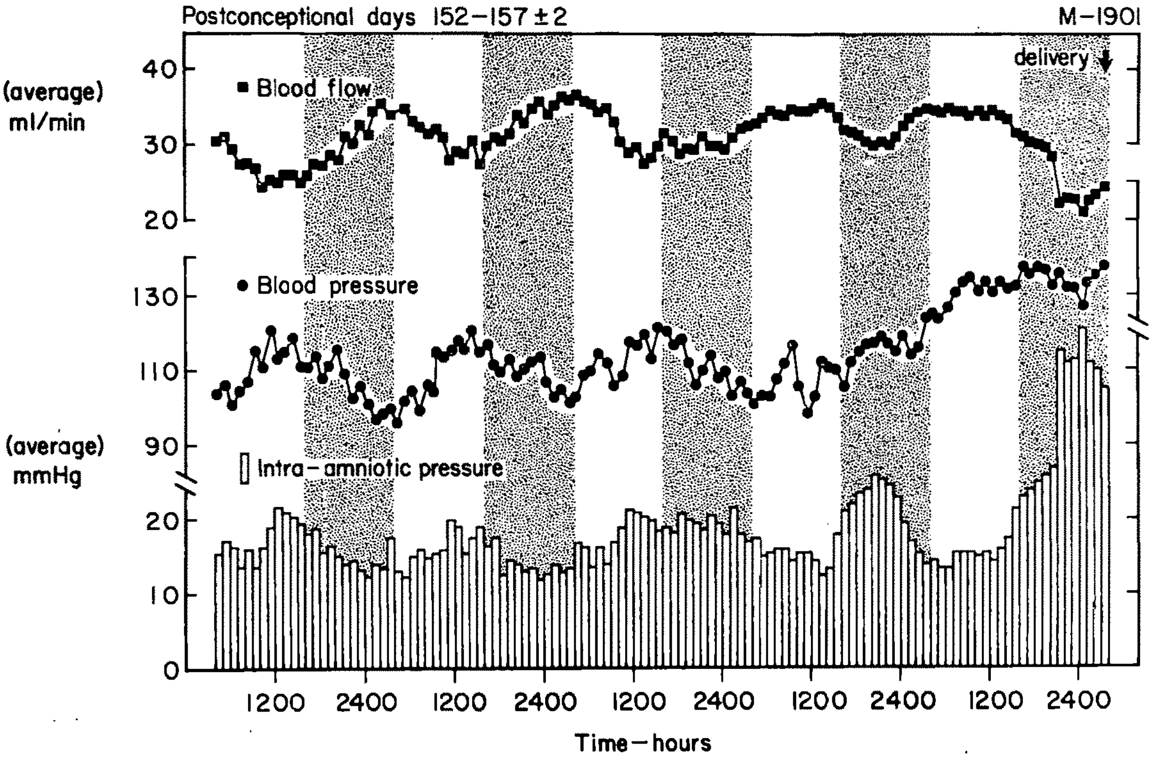

Fig. 5. Illustration of the variations in uterine dynamics recorded over 120 consecutive hours during late pregnancy, labor, and delivery. The linear correlation coefficients calculated by the method of least-squares for comparison of the changes in hourly average values of intra-amniotic pressure with values of blood pressure and flow for corresponding hours were 0.432 and −0.994, respectively. The linear correlation coefficient obtained for comparison of blood flow to blood pressure was −0.398 ($p < 0.05$).

ranged from 83.45 (M-610) to 133.65 (M-609), and multiple correlation coefficients ranged from 0.620 (M-906) to 0.837 (M-010). Regardless of the association of light or dark to the time of delivery, the highest value of the Fourier component occurred during the period of light or early evening hours. The linear portion of the increase in blood pressure values averaged −16.8 mm Hg ± 5.6 and usually began in conjunction with the ascending portion of the Fourier component. Uterine vascular resistance consistently followed a pattern characterized by an exponential increase. The F ratios ranged from 78.27 (M-609) to 91.51 (M-906). Multiple correlation coefficients ranged from 0.787 (M-609) to 0.909 (M-1907). The linear correlation of vascular resistance was more closely related to the changes in intra-amniotic pressures than to the variations in aortic blood pressures.

The 12 hour-periods of light and dark were shifted 180 degrees 5 (M-908) and 8 (M-010) days prior to delivery for two animals. As had been previously observed,[1] the time of maximal and minimal values of the pressure and flow curves shifted to correspond to the new periods of light (1600 to 0400 hours) and dark (0400 to 1600 hours) within 72 hours of the change in light. However, the relationships of the biorhythms of uterine dynamics associated with labor and delivery persisted.

The changes in hourly average values of uterine activity and hemodynamics recorded over 5-day periods from the nine animals in which all sensors functioned satisfactorily throughout labor and delivery are presented as composites in Fig. 6. The data points were compiled from the four monkeys in which delivery occurred during a period of light *(A)* and the five monkeys in which delivery occurred during a period of darkness *(B)*. A sinusoidal pattern was present for all variables. Within both groups, the greatest variability between animals occurred in uterine artery blood flow values, as evidenced by the wider 95% confidence intervals. During the first 3 days illustrated, the mean hourly average values of frequency of contraction of the animals in which delivery occurred during a period

Table II. Periodic regression (24-hour) parameters, second component, and linear regression correlations of uterine activity, uterine artery blood flow, and aortic blood pressure calculated from measurements in 12 monkeys during the 36-hour period terminating in delivery

Animal No.	*Postconceptional age at delivery (days ± 2)*	*Variable**	*Mean*	*Fourier components*		*Second component*	*Time of delivery (hours)*	*Linear correlation coefficient*
				Semi-amplitude	*Acrophase (hours)*			
		Period of light 0400 to 1600 hours; period of dark, 1600 to 0400 hours						
		Delivery occurred during the period of light						
M-906	160	IAP (mm Hg)	17.3	2.2	1016	$0.0020\ e^{(ct)}$	1232	−0.885† (UBF:IAP)
		UCF (contractions/min)	0.65	0.13	1434	0.0481 (ct)		
		UBF (ml/min)	30.6	5.1	2251	$-0.0009\ e^{(ct)}$		−0.599† (UBF:ABP)
		ABP (mm Hg)	116.8	4.0	1346	0.9897 (ct)		0.602† (ABP:IAP)
		UVR (mm Hg · ml^{-1} · min)	3.3	0.8	1102	$0.0003\ e^{(ct)}$		0.951† (UVR:IAP)
M-1905	163	IAP (mm Hg)	16.4	4.8	1102	0.08 (ct)	0806	−0.675† (UBF:IAP)
		UCF (contractions/min)	0.44			0.5890 (ct)		
		UBF (ml/min)	26.3	3.6	2159	$-0.0007\ e^{(ct)}$		
M-1906	151	IAP (mm Hg)	19.5	3.0	1358	$0.0012^{(ct)}$	1506	−0.889† (UBF:IAP)
		UCF (contractions/min)	0.43	0.19	1221	0.0914 (ct)		
		UBF (ml/min)	30.8	5.8	0006	$-0.0008\ e^{(ct)}$		−0.488† (UBF:ABP)
		ABP (mm Hg)	115.7	16.4	1402	2.1 (ct)		0.452† (ABP:IAP)
		UVR (mm Hg · ml^{-1} · min)	3.1	0.5	1431	$0.0002\ e^{(ct)}$		0.966† (UVR:IAP)
M-1908	157	IAP (mm Hg)	16.2	4.2	1040	$0.0019\ e^{(ct)}$	1102	−0.793† (UBF:IAP)
		UCF (contractions/min)	0.52	0.14	1129	0.0618 (ct)		
		UBF (ml/min)	30.0	5.4	0035	$-0.0014\ e^{(ct)}$		−0.642† (UBF:ABP)
		ABP (mm Hg)	105.1	13.7	1043	0.9787 (ct)		0.531† (ABP:IAP)
		UVR (mm Hg · ml^{-1} · min)	2.9	0.6	1041	$0.0003\ e^{(ct)}$		0.874† (UVR:IAP)
		Delivery occurred during the period of dark						
M-609	146	IAP (mm Hg)	13.2	1.9	0141	$0.0010\ e^{(ct)}$	0229	−0.817† (UBF:IAP)
		UCF (contractions/min)	0.49	0.20	2043	0.1085 (ct)		
		UBF (ml/min)	29.6	2.6	0838	$-0.0011\ e^{(ct)}$		−0.278 (UBF:ABP)
		ABP (mm Hg)	114.9	6.6	0821	2.5 (ct)		0.074 (ABP:IAP)
		UVR (mm Hg · ml^{-1} · min)	3.2	0.7	0039	$0.0003\ e^{(ct)}$		0.878† (UVR:IAP)
M-610	159	IAP (mm Hg)	15.7	1.9	2041	$0.0019\ e^{(ct)}$	0044	
		UCF (contractions/min)	0.31			0.0861 (ct)		
		ABP (mm Hg)	108.1	7.7	1016	3.3 (ct)		0.148 (ABP:IAP)
M-1900	161	IAP (mm Hg)	19.5	5.2	1406	$0.0029\ e^{(ct)}$	1804	
		UCF (contractions/min)	0.48			0.0433 (ct)		
M-1907	159	IAP (mm Hg)	19.4	6.7	2118	$0.0031\ e^{(ct)}$	0401	−0.951† (UBF:IAP)
		UCF (contractions/min)	0.39	0.15	1553	0.0445 (ct)		
		UBF (ml/min)	32.5	3.9	0938	$-0.0013\ e^{(ct)}$		−0.254 (UBF:ABP)
		ABP (mm Hg)	109.8	5.1	1540	1.6 (ct)		0.242 (ABP:IAP)
		UVR (mm Hg · ml^{-1} · min)	3.4	0.3	1822	$0.002\ e^{(ct)}$		0.957† (UVR:IAP)
M-1909	157	IAP (mm Hg)	18.3	3.2	0054	$0.0020\ e^{(ct)}$	2400	−0.794† (UBF:IAP)
		UCF (contractions/min)	0.28	0.10	0027	0.0804 (ct)		
		UBF (ml/min)	22.1	2.7	1205	$-0.0009\ e^{(ct)}$		0.761† (UBF:ABP)
		ABP (mm Hg)	113.4	8.8	1238	2.1 (ct)		−0.532† (ABP:IAP)
		UVR (mm Hg · ml^{-1} · min)	2.8	0.4	0031	$0.0002\ e^{(ct)}$		0.884† (UVR:IAP)
M-1950	156	IAP (mm Hg)	16.2	3.4	0341	$0.0025\ e^{(ct)}$	0257	−0.803† (UBF:IAP)
		UCF (contractions/min)	0.35	0.18	0219	0.0374 (ct)		
		UBF (ml/min)	33.7	3.7	1557	$-0.00011\ e^{(ct)}$		0.621† (UBF:ABP)
		ABP (mm Hg)	110.9	9.7	1444	1.9 (ct)		−0.137 (ABP:IAP)
		UVR (mm Hg · ml^{-1} · min)	2.4	0.3	0259	$0.0003\ e^{(ct)}$		0.911† (UVR:IAP)
		Period of light 1600 to 0400 hours: period of dark 0400 to 1600 hours						
		Delivery occurred during the period of light						
M-908	165	IAP (mm Hg)	18.6	2.0	1824	$0.0023\ e^{(ct)}$	2031	−0.782† (UBF:IAP)
		UCF (contractions/min)	0.33	0.11	1118	0.0491 (ct)		
		UBF (ml/min)	37.1	4.9	0926	$-0.0010\ e^{(ct)}$		
		Delivery occurred during the period of dark						
M-010	154	IAP (mm Hg)	16.7	2.6	0703	$0.0030\ e^{(ct)}$	1043	
		UCF (contractions/min)	0.36	0.08	0500	0.0702 (ct)		
		ABP (mm Hg)	112.1	8.2	0237	0.7975 (ct)		0.507† (ABP:IAP)

*IAP = intra-amniotic pressure; UCF = uterine contraction frequency; UBF = uterine artery blood flow; ABP = aortic blood pressure; UVR = uterine vascular resistance.

†$p < 0.05$.

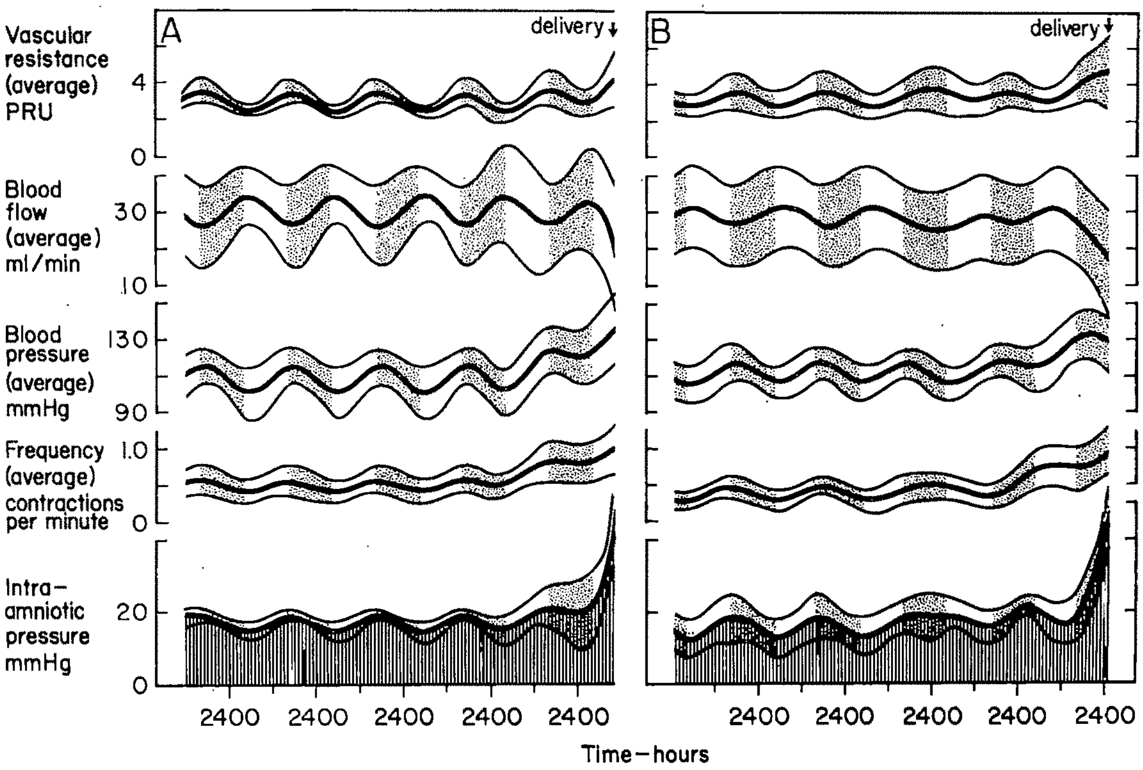

Fig. 6. Composite drawings of the biorhythms of uterine activity and hemodynamics recorded during the 120-hour period terminating in spontaneous labor and delivery. The mean hourly average values of intra-amniotic pressure, frequency of contraction, aortic blood pressure, uterine artery blood flow, and uterine vascular resistance were calculated from continuous recordings made in four monkeys in which delivery occurred during the period of light on postconceptional days 151, 154, 157, and 160 *(A)* and in five monkeys in which delivery took place during the period of darkness on postconceptional days 146, 156, 157, 157, and 159 *(B)*. The narrow outer lines represent the 95% confidence intervals determined for individual mean values. Peripheral resistance units *(PRU)* were calculated by the formula (aortic blood pressure − intra-amniotic pressure) ÷ uterine artery blood flow.

of light were statistically greater than those of animals in which delivery occurred during a period of darkness ($t = 7.77$, $p < 0.001$). The shift in occurrence of maximal values of uterine activity from periods of light to periods of darkness in those animals in which delivery took place during the period of darkness is reiterated.

Comment

Continuous monitoring in the unanesthetized subject allows for observation and interpretation of spontaneous changes in uterine dynamics that may not be appreciated by sporadic recordings or by acute measurements performed in anesthetized subjects. When analyzed as a continuum, the recurring variation in contraction pattern characteristic of spontaneous activity of both human[4] and nonhuman primate[5] uterine muscle has been shown to result in differences in hourly average values of intrauterine pressure and frequency of contraction that modulate with periods of light and dark and can be characterized by statistically significant Fourier curves. In the rhesus monkey, 24-hour biorhythms of hourly average values of intra-amniotic pressure, uterine artery blood flow, and aortic blood pressure are consistent findings during mid and late gestation.[1] The present study demonstrates that these circadian biorhythms in uterine dynamics are incorporated in a distinct and predictable manner into the events characterizing labor and delivery. The patterns of variation associated with parturition can be described statistically (Table I) and are observed to be repetitive for the same monkey during successive labors (Figs. 1 and 2), as well as for labors in other animals (Figs. 3-5). The similarities exist despite differences in the duration of pregnancy and of active labor. The greatest variability in the patterns depends on whether delivery occurred during a period of light or a period of darkness.

The association of the circadian rhythms to the process of labor and delivery is reiterated by the observation that higher hourly average values of uterine activity occurred 24 to 36 hours before delivery for all animals studied (Figs. 1-5). In addition, the transition from minimal to progressively increasing hourly average values of intra-amniotic pressure consistently began during the ascending portion of the Fourier variation in pressure values (Figs. 3 and 4). Delivery also usually occurred close to the highest hourly average value or apogee of the Fourier rhythm in spontaneous uterine activity.

In those animals in which delivery takes place during the period of darkness, parturition appears to override any synchronizing effect of light and dark on the period of maximal uterine activity, and the highest values are observed during the period of darkness (Figs. 2, 3, 5, and 6, *B*). The consistency of this shift in the time of highest value or acrophase of the Fourier rhythm in relation to the periods of light and dark allows its use as an indicator of impending delivery and suggests that specific changes directly related to parturition occur within the maternal organism 2 to 2½ days prior to delivery. Although this relationship was seen only in those animals in which delivery occurred during the periods of darkness, the causative factors may be present also in those animals in which delivery takes place during a period of light. Such presence may explain the statistically higher values of contraction frequency recorded 3 to 5 days prior to parturition when delivery occurs during a period of light (Fig. 6).

The presence of prodromal events is evidenced also in the patterns of frequency of contraction and of aortic blood pressure. However, the factors which begin approximately 24 hours prior to delivery to engender the linear increase in frequency of uterine contractions do not result in detectable concomitant increases in the hour-to-hour variations of intra-amniotic pressure (Fig. 3). Likewise, the progressive increase in blood pressure that begins 24 to 36 hours before delivery is not reflected in the patterns of uterine artery blood flow, intra-amniotic pressure, or uterine vascular resistance (Figs. 4-6). The fact that the circadian pattern of change in aortic blood pressure did not shift in those animals in which delivery occurred during the period of darkness suggests that the major events engendering these prodromal changes are occurring within the uterus rather than systemically.

The chronology of alterations in the biorhythms of uterine dynamics associated with labor and delivery favors a complex mechanism of both steroidal and nonsteroidal effectors in the biochemical regulatory processes. The diurnal variation of spontaneous uterine activity of the nonpregnant uterus is positively correlated with alterations in urinary excretion of norepinephrine in both the human[4] and the nonhuman primate.[6] These associations suggest that catecholamines, specifically norepinephrine, could initiate the cascade of factors involved in the mechanisms of parturition. However, uterine motility is controlled by a variety of biochemical regulatory processes, and the possible influence of norepinephrine on initiation of labor may be indirect rather than direct. Comparison of the F ratios and multiple correlation coefficients obtained by application of the analysis of variance to the hourly average values of uterine activity (Table I) suggests that labor and delivery are not a simple extension or a harmonic of these circadian variations but incorporate other processes, as well.

In the nonpregnant monkey, the Fourier variation of both contraction frequency and intra-amniotic pressure is depressed or abolished by either the depletion of catecholamines or alpha adrenergic blockade.[6] The observation in four of the 18 deliveries that the pattern of frequency of contractions recorded during the last 2 days of pregnancy was not associated with detectable circadian rhythms further suggests that norepinephrine and alpha adrenergic receptors are not the sole biochemical mechanisms responsible for the changes. Alternative possibilities include either a dampening effect on the biorhythms by increased beta adrenergic activity suppressing a detectable amplitude of patterns or the overriding effect of some other substances. Evidence of these possibilities lies in the report that alteration in catecholamine sensitivity is related to progesterone versus estrogen dominance, thus suggesting that the steroid hormones influence either catecholamine receptor concentration or the coupling of receptors to the expression of myometrial function.[7] An increase in the concentration of estrogens in the amniotic fluid, as well as peripheral plasma, has been demonstrated in the rhesus monkey a few days prior to delivery.[8]

Additional substances that may be involved are prostaglandins. Adrenergic nerve stimulation with release of norepinephrine has been demonstrated to induce release of prostaglandins from several organs in various species.[9] Other reports have indicated that prostaglandins of the E and A series, in turn, may influence responses of sympathetic nerve stimulation.[10, 11] Elevation of prostaglandin F in decidua and amniotic fluid of the rhesus monkey preceding and during delivery has been demonstrated.[12] Prostaglandins are also important regulators of blood flow. In general, prostaglandins of both the E and F series not only have a direct action on vascular smooth muscle but also facilitate adrenergic vasoconstriction responses.[13] Prostaglandin E_2 may be involved in mediating the uterine vasodilatation produced by estrogens.[14] Prostacyclin (PGI_2) is a circulating hormone[15] that has been demonstrated to have both vascular and uterine muscle relaxant properties.[16]

Although the precise controlling mechanisms remain obscure, the present study does demonstrate that the processes of labor and delivery in the nonhuman primate follow distinct and predictable patterns that may be manifested by spontaneous changes in the biorhythms of uterine activity initiated 2 to 2½ days before delivery. Further changes take place in the frequency of uterine contraction and aortic blood pressure 24 to 36 hours prior to delivery. These events, as well as the process of labor itself, usually occur in close association with the ascending portion of the circadian rhythm of spontaneous uterine activity, an event known to be associated with norepinephrine and alpha adrenergic receptors in the nonpregnant uterus.[6]

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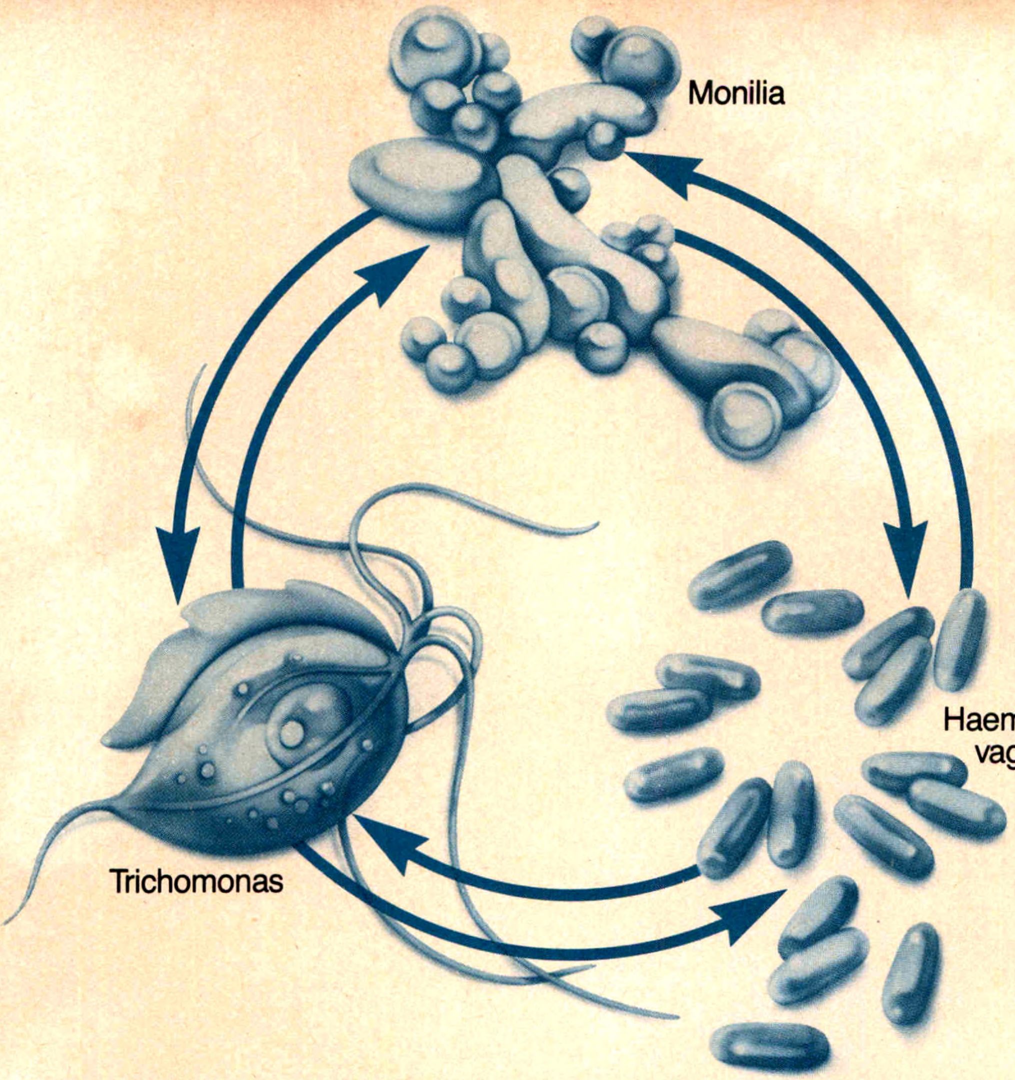

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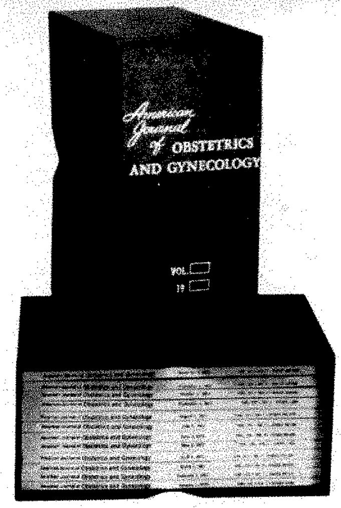

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Effect of blood in amniotic fluid on the detection of phosphatidylglycerol

HOWARD T. STRASSNER, JR., M.D.
STEVEN H. GOLDE, M.D.
GLADYS H. MOSLEY, M.S.
LAWRENCE D. PLATT, M.D.
Los Angeles, California

Samples of amniotic fluid were mixed with serum or red blood cells (RBC) in order to determine the effect of blood contamination on the detection of phosphatidylglycerol (PG). Samples of amniotic fluid were obtained from patients in the second trimester of pregnancy and at term. PG standard was added to half of the aliquots of the second-trimester fluid. The samples of fluid were tested before and after the addition of maternal or fetal serum or RBC in concentrations up to 20%. Two-dimensional thin-layer chromatographic procedures were performed to determine the presence or absence of PG. The results obtained showed that serum or RBC contamination of amniotic fluid without PG did not result in the appearance of a PG spot on the chromatographic plate. Neither serum nor RBC contamination interfered with the detection of PG in those fluids in which it was present. Maternal and fetal blood were similar in their failure to affect the detection of PG. These data suggest that PG determination for fetal lung maturity is a reliable test in the presence of bloody amniotic fluid. (AM. J. OBSTET. GYNECOL. 138:697, 1980.)

SINCE Gluck and associates[1] introduced the lecithin/sphingomyelin (L/S) ratio, it has become the standard test for the assessment of fetal lung maturity, and a large body of literature has documented its usefulness and reliability. Similarly, the rapid surfactant test ("shake test"), described by Clements and associates,[2] has also found wide use and acceptance as a predictor of fetal lung maturity. Recently, the presence in amniotic fluid of phosphatidylglycerol (PG), as detected by two-dimensional thin-layer chromatography, has been shown to correlate well with the presence of fetal lung maturity and the absence of respiratory distress syndrome in the neonate.[6-8]

Both the L/S ratio and the shake test suffer a disadvantage, that is, when amniocentesis is complicated by blood contamination of the amniotic fluid, the tests either may be invalid or interpretation of them may be compromised because of the presence of the blood.[3-5] However, PG is present in relatively large amounts only in the lung and in lung surfactant,[9, 10] whereas in other mammalian tissues it is either absent or present only in minute amounts.[11] Therefore, the suggestion has been made,[6, 8, 12, 13] but not substantiated, that PG determination for fetal lung maturity may be reliable and, consequently, the method of choice when blood contamination of amniotic fluid occurs. The present study was designed to evaluate that hypothesis, specifically, to ascertain the effect of contamination of amniotic fluid by blood of maternal or fetal origin on the subsequent determination of PG.

Material and methods

Uncontaminated amniotic fluid was obtained from patients who were undergoing amniocentesis late in the second trimester of pregnancy or at term. Maternal blood was obtained by venipuncture, and fetal blood was obtained from the umbilical cord immediately after delivery. The cellular and serum fractions of the blood were separated by centrifugation, and red blood cell (RBC) and serum contaminations were carried out

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine, and Women's Hospital, Los Angeles County/University of Southern California Medical Center.

Supported in part by National Institutes of Health Grant No. HD07086-04.

Presented at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980.

Reprint requests: Howard T. Strassner, M.D., Department of Obstetrics and Gynecology, Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, 1753 W. Congress Pkwy., Chicago, Illinois 60612.

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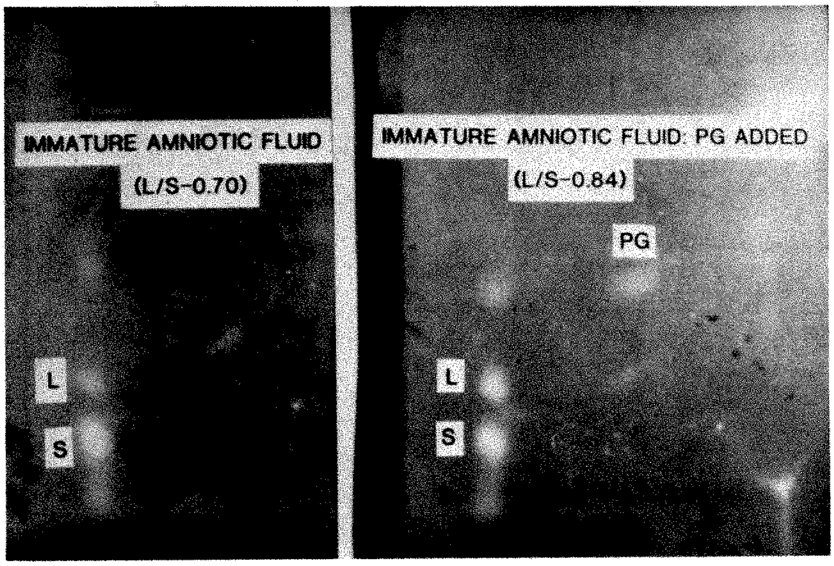


Fig. 1. Two-dimensional thin-layer chromatograms of second-trimester amniotic fluid alone *(left)* and of the same fluid with PG standard added *(right)*.

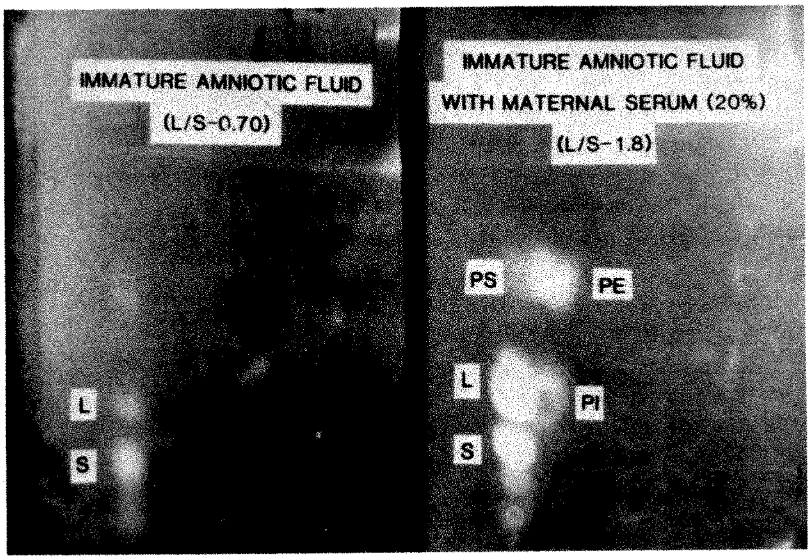
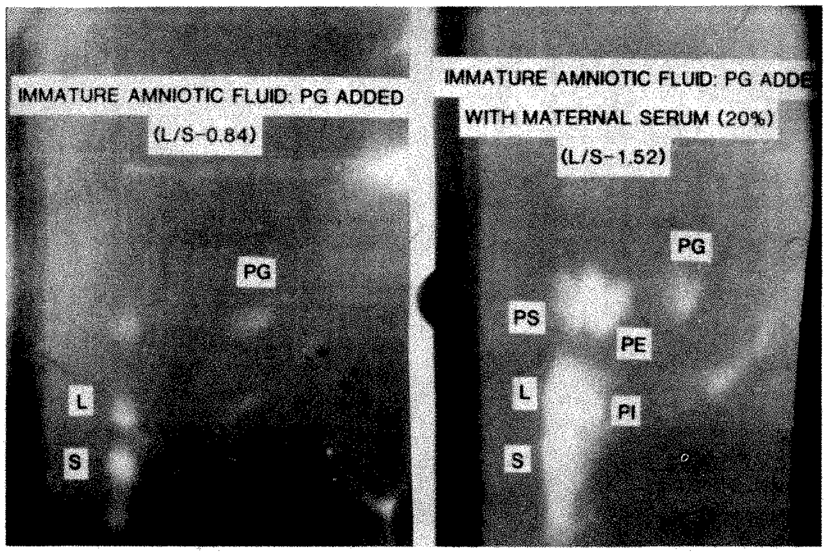


Fig. 2. Two-dimensional thin-layer chromatograms of second-trimester amniotic fluid alone *(left)* and of the same fluid after contamination with maternal serum in 20% concentration *(right)*.

separately. Specimens of blood for the RBC contamination experiments were collected in heparinized tubes, and the cells were washed with saline solution prior to use.

For each experiment, the samples of second-trimester amniotic fluid were pooled to provide sufficient volume and then divided into several aliquots. PG standard (Sigma Chemical Company) was mixed with half the aliquots. The aliquots with and without the PG standard were then contaminated with either maternal serum, maternal RBC, fetal serum, or fetal RBC in concentrations from 1% to 20%. Uncontaminated samples with and without added PG served as controls for comparison with the contaminated specimens. Samples of amniotic fluid were also obtained from patients at term and were tested without contamination and after contamination with maternal serum, maternal RBC, fetal serum, or fetal RBC in concentrations from 1% to 20%. All specimens were prepared and tested in duplicate.

The procedures for PG determination were then carried out. All samples were centrifuged (1,100 × g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(L/S-0.84)
PG
L
S
IMMATURE AMNIOTIC FLUID: PG ADDE
WITH MATERNAL SERUM (20%)
(L/S-1.52)
PG
PS
PE
L
PI
S

Fig. 3. Two-dimensional thin-layer chromatograms of second-trimester amniotic fluid with PG standard added *(left)* and the same fluid with PG standard and maternal serum in 20% concentration *(right)*.

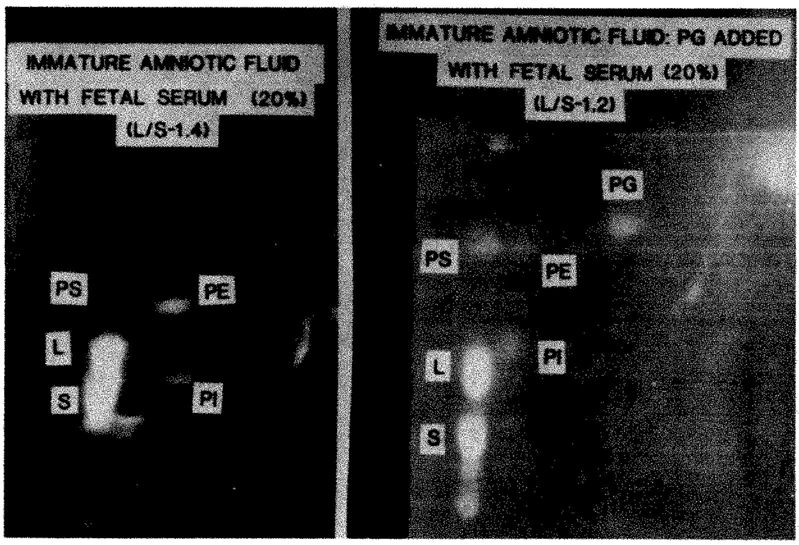

Fig. 4. Two-dimensional thin-layer chromatograms of second-trimester amniotic fluid with 20% concentration of fetal serum alone *(left)* and the same fluid with PG standard added and 20% concentration of fetal serum *(right)*.

for 10 minutes) prior to processing and the fluid was decanted. Amniotic fluid lipid extraction and two-dimensional thin-layer chromatography were then performed by the methods previously described for our laboratory.[8] PG was identified on the chromatographic plate by its characteristic spot separation pattern.

Results

The samples of uncontaminated second-trimester amniotic fluid all had immature L/S ratios, and they did not yield a PG spot on the chromatographic plate. When these uncontaminated fluids were mixed with PG standard, a PG spot appeared on the chromatogram in its characteristic location (Fig. 1).

A PG spot was never produced by serum contamination of the amniotic fluid that did not contain PG (Fig. 2). In addition, serum contamination did not interfere with the detection of the PG spot in those fluids to which PG had been added (Fig. 3). Maternal and fetal serum contamination produced similar results (Fig. 4).

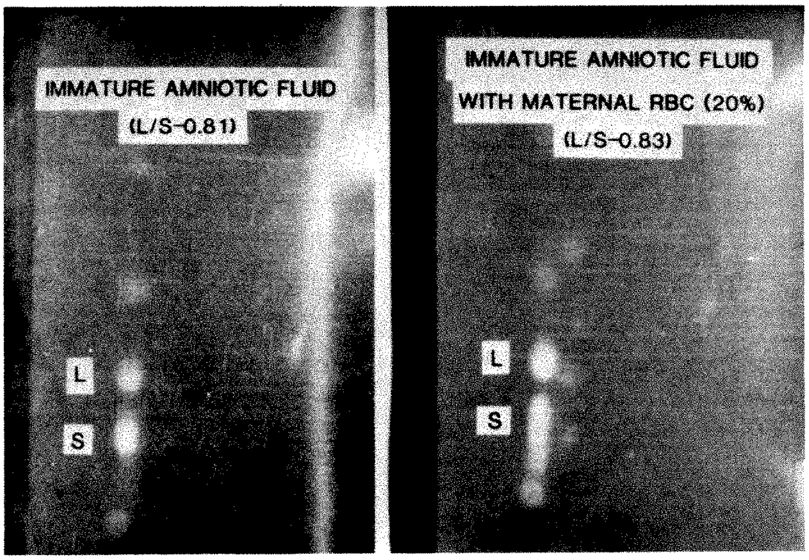

Fig. 5. Two-dimensional thin-layer chromatograms of second-trimester amniotic fluid alone *(left)* and the same fluid after contamination with maternal RBC in 20% concentration *(right)*.

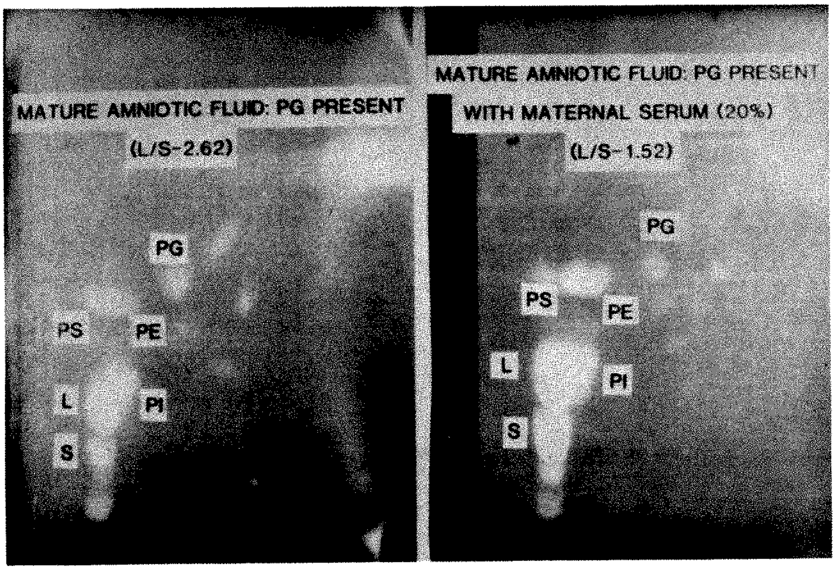
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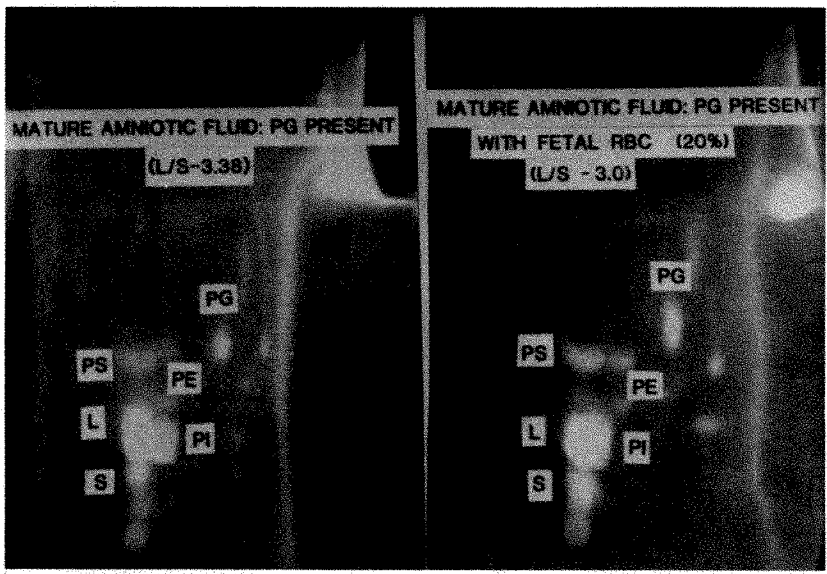
Fig. 6. Two/dimensional thin/layer chromatograms of amniotic fluid from a patient at term with naturally occurring PG *(left)* and the same fluid after contamination with maternal serum in 20% concentration *(right)*.

Serum contamination of the immature second-trimester fluids did result in increasing L/S ratios with increasing serum concentration, although they never reached mature values. Also, increasing serum contamination produced or increased the phospholipid spots in locations characteristic for phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidylethanolamine (PE) (Figs. 2 and 3).

Neither maternal nor fetal RBC contamination of the second-trimester amniotic fluid produced a PG spot from those fluids that did not contain the PG standard. The RBC contamination also did not interfere with the detection of PG in those fluids to which PG had been added. Furthermore, RBC contamination did not significantly affect the L/S ratio or the phospholipid pattern of the immature fluids (Fig. 5).

In these experiments, all of the samples of uncontaminated amniotic fluid obtained from patients at term had mature L/S ratios and naturally occurring PG. Neither maternal nor fetal serum contamination of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PS
PE
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PI
S

Fig. 7. Two-dimensional thin-layer chromatograms of amniotic fluid from a patient at term with naturally occurring PG *(left)* and the same fluid after contamination with fetal RBC in 20% concentration *(right)*.

the amniotic fluid prevented the detection of PG (Fig. 6). Furthermore, contamination of these samples of fluid with RBC, whether of maternal or fetal origin, again had no affect on the detection of PG (Fig. 7). However, the L/S ratio was lowered with increasing serum contamination and did reach immature values. RBC contamination did not significantly affect the L/S ratio.

Comment

Amniocentesis for the purpose of determining fetal lung maturity has become an accepted and widely used obstetric procedure. Ultrasound examination for localization of the fetus and placenta prior to amniocentesis has reduced, but certainly not eliminated, the occurrence of blood contamination of the amniotic fluid obtained.[14–16]

The effect of blood contamination of amniotic fluid on the determination of the L/S ratio has been systematically evaluated by Buhi and Spellacy,[4] who reported that high L/S ratios were decreased and low L/S ratios were elevated after contamination of amniotic fluid with either maternal or fetal serum. Our findings support theirs. Keniston and associates[5] have similarly evaluated the effect of blood contamination of amniotic fluid on the rapid surfactant test, and found that false predictions of maturity may result from such contamination. It is apparent, then, that both of the tests of fetal lung maturity currently in widest use may be significantly compromised by blood contamination of amniotic fluid.

Published clinical studies have indicated that the detection of PG in amniotic fluid by two-dimensional thin-layer chromatography reliably and specifically predicts functional lung maturity.[6–8] The present study aimed to determine whether the presence of blood in amniotic fluid would compromise the reliability of this promising new method, and the findings showed that blood contaminants in immature amniotic fluid did not produce a PG spot. Moreover, blood contaminants did not interfere with the detection of PG when it was present. Both maternal and fetal blood similarly failed to compromise the test. Therefore, the determination of PG should remain a reliable procedure in the presence of bloody amniotic fluid, and may become the procedure of choice for evaluating fetal lung maturity when blood contamination of amniotic fluid occurs.

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Umbilical venous blood flow and its distribution before and during autonomic blockade in fetal lambs

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In nine chronically catheterized fetal lambs (120 to 135 days; term 147 days), umbilical venous blood flow and its distribution were measured by the radionuclide-labeled microsphere technique before and during autonomic blockade with atropine (0.20 to 0.25 mg/kg; seven studies) or phentolamine (0.10 to 0.12 mg/kg; six studies). Atropine significantly increased mean fetal heart rate (182 to 207 beats/min), descending aortic blood pressure (49 to 55 mm Hg), and umbilical venous blood flow (210 to 239 ml/min/kg fetus), without changing umbilical venous blood pressure. Phentolamine decreased mean descending aortic pressure (48 to 45 mm Hg), but did not affect heart rate, umbilical venous blood pressure, or umbilical venous blood flow. Neither atropine nor phentolamine altered the distribution of umbilical venous blood flow to the ductus venosus, the liver, or the other fetal organs. These data indicate that the cholinergic nervous system only indirectly affects the basal umbilical venous blood flow in the near-term fetal lamb. This effect is small and is secondary to associated changes in heart rate and arterial blood pressure. Neither the cholinergic nor the α-adrenergic systems influence the basal distribution of umbilical venous blood flow.
(AM. J. OBSTET. GYNECOL. 138:703, 1980.)

UMBILICAL venous blood returning to the fetus either is distributed to the liver or is shunted through the ductus venosus to supply the other fetal organs and body.[1, 2] The mechanisms that regulate distribution of umbilical venous blood flow are only partially understood.[3-8] Histochemical and cineangiographic investigations in the lamb[3, 4] and human fetus[6, 7] have suggested that the autonomic nervous system affects the shunting of umbilical venous blood flow through the ductus venosus. However, no studies have quantitatively assessed the basal influence of the autonomic nervous system on the distribution of umbilical venous blood flow in the intact unstressed fetus. In the present experiments, the effects of cholinergic and α-adrenergic blockade on umbilical venous and ductus venosus blood flows and distributions were determined. Cholinergic blockade with atropine and α-adrenergic blockade with phentolamine were produced in chronically catheterized fetal lambs which were remote from the surgical and anesthetic influences that can affect the systemic and umbilical circulations.

Methods

Nine fetal lambs of known gestational ages were studied; gestational ages at surgical preparation ranged from 118 to 133 days (term = 147 days). After spinal or epidural anesthesia was induced in each ewe with tetracaine HCl, 1% (Pontocaine, Winthrop), the maternal abdomen and pregnant uterine horn were opened and polyvinyl catheters were placed in a fetal hindlimb vein and in two hindlimb arteries; these catheters were advanced into the inferior vena cava and descending aorta, respectively.[1] Analgesia for

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Presented at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980.

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**Supported by a Basil O'Connor Starter Research Grant (#5-225) from the March of Dimes Birth Defects Foundation.*

***Supported by a Student Summer Research Fellowship provided by the Medical Alumni Association of the University of Pittsburgh.*

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placement of the catheters was provided by local infiltration of lidocaine HCl, 0.5% (Xylocaine, Astra). Two catheters were placed in cotyledonary tributaries of an umbilical vein and were advanced 10 to 15 cm so that the tips of the catheters were in a main umbilical vein.[8] (All catheter positions were verified at postmortem examination.) A catheter was placed in the amniotic sac, the uterine incision was closed, and the catheters were brought out subcutaneously to the ewe's flank, where they were stored in a canvas pouch.

Two to 4 days postoperatively, the fetuses of the unanesthetized ewes were studied as the ewes stood unrestrained in an open transport cage. Continuous measurement was made of fetal descending aortic, inferior vena caval, and umbilical venous blood pressures, amniotic fluid pressure, and heart rate, and these were recorded on a direct-writing recorder (Beckman Dynograph R611). Blood pressures were obtained with pressure transducers (Statham P23Db) and were corrected to a common reference point (intrauterine pressure) by subtracting the simultaneously measured amniotic fluid pressure. Samples of descending aortic and umbilical venous blood were obtained for analysis of blood gases and pH. Po_2, Pco_2, and pH were analyzed at 37° C with standard electrodes and a blood gas meter (Corning 165).

After stabilization of the circulatory variables had been accomplished, radionuclide-labeled microspheres (15 μm diameter, selected from the following: ^{125}I, ^{141}Ce, ^{51}Cr, ^{85}Sr, ^{95}Nb, or ^{46}Sc—3M Company, New England Nuclear) were injected into an umbilical venous catheter while a reference sample of blood was withdrawn from the other descending aortic catheter.[9] The volume of blood removed in the reference sample (about 10 ml) was replaced with maternal venous blood. Then, either atropine, 0.20 to 0.25 mg/kg estimated fetal body weight (seven studies), or phentolamine (Regitine, Ciba), 0.10 to 0.12 mg/kg (six studies), was administered into an umbilical vein while heart rate and arterial and venous blood pressures continued to be recorded. Atropine or phentolamine was prepared freshly in 2-ml saline solution and was infused over approximately 1 minute. The amount of each drug injected for blockade was based on previous investigations[10, 11]; doses were related to fetal body weight as estimated at the time of surgical preparation. Completeness of cholinergic or α-adrenergic blockade was established by the absence of changes in heart rate or blood pressure in response to the administration of the appropriate agonist (acetylcholine, 10 μg/kg, or methoxamine, 50 μg/kg[10]) into an umbilical vein. Approximately 2 minutes after autonomic blockade was confirmed, microspheres labeled with a different radionuclide were injected into an umbilical vein while a second reference sample of blood was being withdrawn from the descending aorta. Another set of determinations of descending aortic and umbilical venous blood gases and pH was also obtained.

Fetal arterial and venous blood pressures and heart rates were recorded during all injections of microspheres and withdrawals of reference samples of blood in order to ensure that the circulation remained in a steady state during the measurements of blood flow. Fetal response to only one blocking agent was studied per day; a maximum of only two studies was performed in each fetus.

At the end of the studies, the ewe and fetus were killed with sodium pentobarbital. The entire uterus and its contents were removed, each fetus was dissected uniformly, and all animal tissues were analyzed. Left and right kidneys were analyzed separately in order to assess the adequacy of mixing of microspheres in the distal aortic bloodstream.[9, 12] The amount of each radionuclide in each tissue sample was measured with a well-type gamma scintillation counter and a multichannel pulse-height analyzer (Inotech, Inc., Searle Analytical Co.).[9] Reference samples of blood and fetal organs contained more than 1,500 microspheres. Thus, the maximum error in any measurement of blood flow or distribution was 5% at the 95% confidence limits.[12]

The methods for calculating umbilical venous blood flow and its distribution to liver, ductus venosus, and the other fetal organs have been described in detail.[1, 2, 8] In summary, the portion of the total radionuclide injected into an umbilical vein that was trapped in the liver represented the fraction of umbilical venous blood flow that supplied the liver ($f_{UV(liver)}$). The remainder of total umbilical venous blood flow entered the ductus venosus to be distributed to the fetal organs, body, and placenta. Total umbilical venous blood flow (Q_{UV}) was assumed to be equal to the arterial blood flow to the placenta and was calculated by the reference blood flow technique[9]: Q_{UV} = (cpm placenta/cpm reference sample) (reference sample blood flow), where cpm = radioactive counts/min; reference sample blood flow was determined by calibrating the withdrawal pump. Umbilical venous blood flow to the liver ($Q_{UV(liver)}$) = (Q_{UV}) ($f_{UV(liver)}$), and umbilical venous blood flow through the ductus venosus ($Q_{UV(DV)}$) = $Q_{UV} - Q_{UV(liver)}$. A small error in $Q_{UV(DV)}$ was made by not including in this calculation the microspheres that entered the ductus venosus and subsequently reached the liver via the hepatic artery. This error averaged approximately 1%.[1] The above calculations require that microspheres injected into an umbilical vein adequately mix with umbilical venous blood before reach-

Table I. Heart rate, blood pressures, and umbilical venous blood flows before and during autonomic blockade

	Atropine		*Phentolamine*	
	Before	*During*	*Before*	*During*
n (studies)	7		6	
Gestational age (days)	127 ± 5		126 ± 5	
Fetal body weight (kg)	2.9 ± 0.5		2.9 ± 0.5	
Heart rate (bpm)	182 ± 11	207 ± 16*	168 ± 9	174 ± 13
Mean blood pressures (mm Hg)				
Descending aortic	49 ± 8	55 ± 10*	48 ± 6	45 ± 5*
Umbilical venous	8 ± 2	8 ± 1	9 ± 3	8 ± 3
Inferior vena caval	3 ± 2	3 ± 1	4 ± 1	3 ± 2
Mean blood flows (ml/min/kg)				
Umbilical venous (total)	210 ± 15	239 ± 19*	219 ± 18	233 ± 35
Umbilical venous flow to ductus venosus	101 ± 26	122 ± 56	114 ± 38	126 ± 22
Umbilical venous flow to liver	109 ± 22	117 ± 47	105 ± 20	107 ± 30

Values are means ± 1 SD.
*$p < 0.05$, two-tailed paired t test.

Table II. Distribution (%) of umbilical venous blood flow to fetal organs and body before and during autonomic blockade

	Atropine		*Phentolamine*	
	Before	*During*	*Before*	*During*
Liver	51.8 ± 9.0	48.8 ± 20.9	48.8 ± 12.8	45.6 ± 9.8
Brain	2.8 ± 1.2	3.2 ± 2.1	3.2 ± 1.5	3.3 ± 1.5
Heart	2.0 ± 0.3	3.1 ± 1.2	2.1 ± 1.7	2.4 ± 1.3
Lungs	1.2 ± 0.8	1.9 ± 1.2	3.5 ± 2.8	3.1 ± 2.5
Gut and spleen	3.1 ± 0.9	3.2 ± 1.1	3.8 ± 2.1	3.9 ± 1.6
Kidneys	1.0 ± 0.5	1.1 ± 1.0	1.0 ± 0.3	0.9 ± 0.2
Body	22.7 ± 2.5	22.2 ± 4.7	19.6 ± 4.1	20.3 ± 2.1
Placenta	15.5 ± 5.2	16.6 ± 11.1	18.0 ± 3.5	20.5 ± 3.8

Values are mean ±1 SD; n, gestational ages, and fetal weights for the atropine and phentolamine studies are as in Table I.

ing the liver or the ductus venosus, which has been previously verified.[2, 8]

All values presented in the text and tables are means ± 1 SD. Each fetus served as its own control. The two-tailed paired t test was used to compare values obtained before and during blockade of the autonomic nervous system. Since the blood flow distributions, which were percentages, formed a binomial distribution rather than a normal distribution, these data were first transformed with an arcsine transformation to produce a normal distribution for statistical analysis. P values <0.05 were considered to be significant.[13]

Results

Fetal heart rate, arterial and venous blood pressures, blood gases and pH, and umbilical venous blood flow and its distribution obtained before administration of atropine (n = 7) did not differ significantly from those values obtained before administration of phentolamine (n = 6) (analyzed by t test of the group means). Gestational ages and fetal weights in the two series of studies were the same (Table I). Blood gases and pH in blood from the descending aorta for the combined series of control studies (n = 13) were: pH 7.37 ± 0.05; Po_2 19 ± 1 torr; and Pco_2 43 ± 2 torr. Umbilical venous blood gases and blood pH were: pH 7.45 ± 0.02; Po_2 26 ± 1 torr; and Pco_2 39 ± 2 torr.

Table I shows that cholinergic blockade with atropine increased fetal heart rate and mean descending aortic blood pressure without changing umbilical venous or inferior vena caval blood pressures. Total umbilical venous blood flow increased significantly (approximately 14%), although there were no significant changes in umbilical venous flows through the ductus venosus or to the liver. Alpha-adrenergic blockade with phentolamine did not affect heart rate or umbilical venous and inferior vena caval blood pressures, but did decrease the mean descending aortic pressure. Total umbilical venous blood flow and the umbilical flows to ductus venosus and liver were not altered significantly.

Table II shows that neither atropine nor phentolamine changed the distribution of umbilical venous blood flow to the various fetal organs. Within the narrow gestational age range studied (120 to 135 days), there were no obvious differences in vascular responsiveness to autonomic blockade due to increases in gestational age or fetal weight. Descending aortic and umbilical venous blood gases and pH were unaffected by the administration of either atropine or phentolamine.

The mean difference in radioactive counts per minute per 100 grams of tissue between the left and right kidneys was less than 8% either before or during autonomic blockade with atropine or phentolamine, thus indicating adequate mixing of microspheres in the distal aortic bloodstream during each study.

Comment

Histochemical studies have identified cholinergic and adrenergic neural fibers in the intra-abdominal portion of the umbilical vein and in the fetal ductus venosus. In the lamb fetus, Barron[3] found that the smooth muscle of the ductus venosus was innervated only by nerve fibers from the two vagi. Ehinger and associates[6] identified only adrenergic innervation in the intra-abdominal umbilical vein and in the ductus venosus of the human fetus, whereas Pearson and Sauter[7] recognized both cholinergic and adrenergic nerve fibers. The cineangiographic studies of Arstila and associates[4] in lambs suggested that the ductus venosus contained cholinergic and adrenergic receptors which, when stimulated, resulted in its dilation. Arstila and associates,[4] Ehinger and associates,[6] and Pearson and Sauter[7] speculated that either the parasympathetic or the sympathetic nervous systems, or both, directly influenced ductus venosus blood flow. However, their studies, did not indicate whether blockade of the autonomic nervous system affected distribution of umbilical venous blood flow to the ductus venosus or the various vital organs of the intact fetus in utero.

The present investigations showed that the autonomic nervous system only minimally affected basal umbilical venous blood flow or its distribution in the undisturbed near-term fetal lamb. Selective blockade of the cholinergic nervous system with atropine increased fetal heart rate and descending aortic blood pressure, changes that were consistent with those of previous studies.[10, 11] Since umbilical blood flow correlates positively with fetal heart rate and arterial blood pressure,[14] increases in heart rate or descending aortic pressure induced by cholinergic blockade might be expected to secondarily increase umbilical venous blood flow. Thus, our finding of a 14% mean increase in umbilical venous blood flow during cholinergic receptor blockade was probably due secondarily to increases in arterial blood pressure and fetal heart rate, rather than to specific effects of parasympathetic blockade on the umbilical vein, the circulation in the liver, or the ductus venosus. Nonetheless, it is possible for cholinergic blockade to influence the vasculature of the liver and the ductus venosus directly. McCuskey[5] demonstrated that blood vessels in the fetal rabbit liver dilated in response to topical administration of acetylcholine, the cholinergic neurotransmitter, thereby indicating that the vasculature of the liver contained cholinergic receptors. In the lamb, Arstila and associates[4] found that either the administration of acetylcholine or vagal stimulation caused dilation of the ductus venosus. These two studies[4, 5] suggested that parasympathetic stimulation produced increased blood flow to both the liver and the ductus venosus. Since autonomic blockade reflects withdrawal of the influence of the autonomic nervous system, cholinergic blockade should, if anything, decrease flow through these two circulations; our findings indicated no such influence.

Alpha-adrenergic blockade with phentolamine did not change heart rate or umbilical blood flow but did result in a small decrease in descending aortic mean blood pressure, findings that have been reported previously.[10, 11, 15] Since there was little absolute change in heart rate or arterial pressure, total umbilical blood flow would not be expected to change significantly,[14] unless autonomic blockade influenced the umbilical or liver circulations or the ductus venosus directly. Although there are few, if any, adrenergic nerve fibers or receptors in the umbilical circulation,[6, 14] adrenergic nerves are present in the fetal liver circulation[5] and in the ductus venosus.[6, 7] Our data, however, show that adrenergic innervation of the liver and ductus venosus does not contribute significantly to the resting tone of these circulations.

In conclusion, our studies indicate that the cholinergic nervous system only indirectly influences the basal umbilical venous blood flow in the near-term fetal lamb. This influence is small and is secondary to concomitant changes in heart rate and aortic blood pressure. Nonetheless, blockade of either the cholinergic or the α-adrenergic system does not affect the pattern of distribution of umbilical venous blood flow in the intact unstressed fetus in utero.

We wish to thank Anthony Battelli and Amy Ingraham for their skillful technical assistance and Susan Gates for assistance in preparation of the manuscript.

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Suppression of human chorionic gonadotropin by progestational steroids

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The dynamics of the secretion of human chorionic gonadotropin (hCG) were studied by culturing explants of normal term placentas for as long as 144 hours. A significant accumulation of immunoreactive hCG (β-subunit) was first detected at 48 to 72 hours, and a sixfold increase in hCG was observed in control culture medium at 144 hours. Compared to control cultures, progesterone ($P < 0.001$) in physiologic tissue levels of 5 to 20 μg/ml, pregnenolone ($P < 0.001$), 20 μg/ml, and 20α-dihydroprogesterone ($P < 0.001$), 20 μg/ml, suppressed the secretion of hCG throughout the study period. Progesterone decreased the secretion of hCG in a dose-response manner ($r = -0.8S87$, $P < 0.1$). No suppression of hCG was observed in the presence of cortisol, testosteorne, dihydrotestosterone, 17β-estradiol, or estriol. The secretion of human chorionic somatomammotropin was unchanged in the presence of progesterone. The augmented hCG response in the presence of dibutyl cAMP ($P < 0.001$) was significantly, but not completely, suppressed by progesterone 20 μg/ml culture medium ($P < 0.01$). Under these conditions, progestational steroids or their immediate metabolites suppress the secretion of hCG, and they may be responsible for the decline in the levels of hCG during pregnancy. (AM. J. OBSTET. GYNECOL. 138:708, 1980.)

THE HUMAN PLACENTA has been considered to be autonomous in its synthesis and secretion of protein and steroid hormones, but the patterns of individual hormone secretion differ during pregnancy. The secretion of human chorionic gonadotropin (hCG) differs from that of other placental hormones by reaching peak concentrations in maternal[1] and fetal[2] plasma during the first trimester and then decreasing to relatively low levels for the remainder of gestation. Isolation of the messenger-RNA (mRNA) for the alpha and beta subunits of hCG has helped to explain the pattern of hCG secretion. The placental concentrations of alpha and beta subunits of hCG throughout pregnancy parallel the concentrations of these subunits in plasma. Daniels-McQueen and co-workers[3] found that mRNA isolated from first-trimester placenta directed the synthesis of eightfold more alpha subunit in wheat germ cell-free systems than did RNA obtained from term placenta; no mRNA for the β-subunit was detected from term placenta. These findings indicate that the synthesis of the β-subunit may be the rate-limiting step in the secretion of hCG in vivo, but the factors which control the synthesis and secretion of the β-subunit or the intact hCG hormone are basically unknown.

Progesterone is the principal placental steroid, and the secretion of it is necessary for the maintenance of pregnancy. Most of the biologic activities attributed to progesterone are manifested by induction or inhibition of protein synthesis. The concentration of progesterone increases throughout pregnancy in both fetal[4] and maternal plasma.[5] The production of progesterone by the corpus luteum is stimulated by hCG, but the placenta is the major soource of progesterone after the first 7 weeks of gestation.[6] Concentrations of progesterone in placental tissue as high as 10 μg per gram of tissue have been measured,[7] and the levels in the syncytiotrophoblast, the cell of origin of both hCG and progesterone, are likely to be even higher. In these concentrations, progesterone could modulate the synthesis of hCG within the syncytiotrophoblast.

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Presented at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980.

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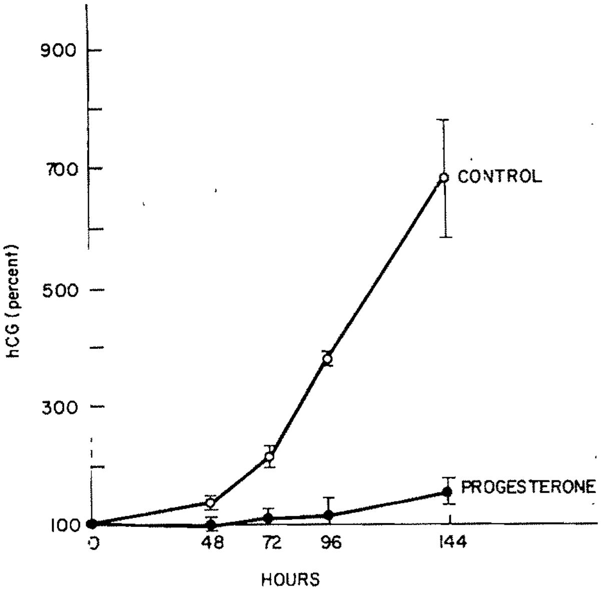

Fig. 1. Secretion of hCG (mean ± SEM) by control and progesterone-treated placental explants, expressed as percent increase in hCG from baseline tissue concentrations.

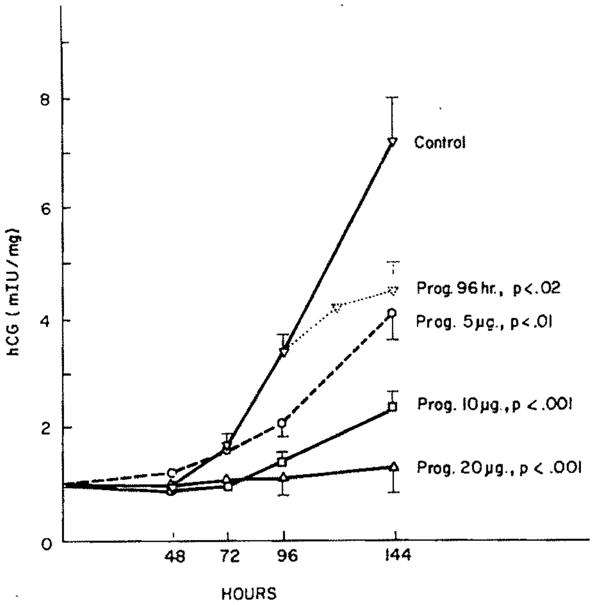

Fig. 2. Secretion of hCG (mean ± SEM) was significantly decreased from control levels by progesterone 5 to 20 μg/ml culture medium and by progesterone 20 μg/ml added to the medium at 96 hours incubation after the initial increase in hCG.

The purpose of this investigation was to study the control of hCG synthesis and secretion by examining the effects of progesterone and other progestational steroids on the secretion of hCG by placental tissue in organ culture.

Methods and material

Term placental tissue was obtained from seven patients who were undergoing elective repeat cesarean section prior to the onset of labor. Villous tissue, free of chorionic membranes, was immediately placed in McCoy's 5a medium for transport, and was prepared for organ culture within 1 hour. Under sterile technique, fronds of placental villi (3 to 5 mm in diameter) were washed three times in McCoy's 5a medium, and two to three pieces (10 to 20 mg) were randomly selected and placed in 35 mm Falcon culture dishes. Cultures were maintained for 96 to 144 hours in a 95% air–5% CO_2 incubator at 37° C.

Culture medium. Each set of tissue was cultured separately in 2 ml of McCoy's 5a medium (modified, GIBCO), containing 2.0 mM L-glutamine (GIBCO) and 10% fetal calf serum (GIBCO). Each set of treated tissues was incubated in quadruplicate and compared to eight sets of control tissues. The culture media from control and treated plates were collected and replaced at 48, 72, and 96 hours. The experiments were terminated at 144 hours, the media were collected and stored at −20° C until analyzed, and the wet tissue weights were recorded.

The placental explants were also cultured in the presence of one or more of the following agents: 1 to 20 μg of progesterone (P) per milliliter of culture medium; 20 μg/ml of pregnenolone (Preg), 17α-hydroxyprogesterone (17-OHP), 20α-dihydroprogesterone (20α-P), testosterone (TO), dihydrotestosterone (DHT), 17β-estradiol (E_2), estriol (E_3), cortisol (F); 10^{-4} to 10^{-6}M aminoglutethimide (CIBA); 5 × 10^{-4}M WIN-24540 (Sterling-Winthrop); 1.0 mM dibutyl cyclic adenosine monophosphate (bu_2cAMP, Sigma). In one experiment, 20 mg of P was added to each milliliter of culture medium at 96 hours. All steroids were obtained from Steraloids, Inc., Wilton, New Hampshire, and they were purified by celite chromatography prior to addition to the culture medium.

Radioimmunoassay procedures. The β-subunit of hCG was measured in control and treated cultures by specific radioimmunoassay with the use of polyethylene glycol (PEG) separation (modified from Serono Laboratories, Boston, Massachusetts). After an incubation period of 18 to 24 hours, 0.1 ml of hCG-free pooled male and female serum, diluted 1:6 with assay buffer, was added to each assay tube (except the total count) to facilitate separation. After PEG separation, the antigen-antibody complex was rinsed with 1.0 ml of cold distilled water to minimize the effect of nonspecific binding. In media that contained small concentrations of hCG, the hCG standards were reconstituted in for-

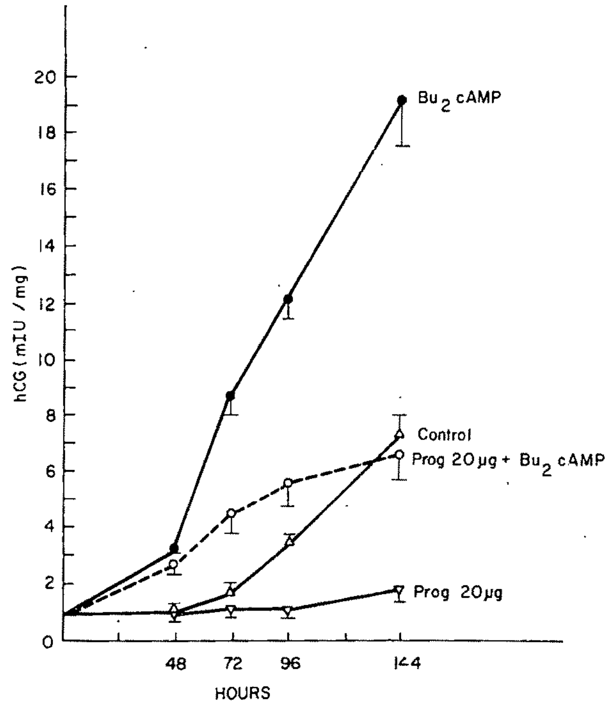

Fig. 3. Effect of progesterone 20 μg/ml culture medium on the bu$_2$cAMP-augmented hCG synthesis (mean ± SEM).

tified McCoy's 5a medium in order to assure uniformity in protein concentration. All assays were performed in duplicate, and the assay sensitivity with this protocol was 3.0 mIU/ml. The intra-assay and interassay coefficients of variation were less than 7%. The results are expressed in mIU βhCG/mg of tissue.

Concentrations of human chorionic somatomammotropin (hCS) in culture medium were measured by specific radioimmunoassay (modified from Amersham Corporation, Arlington Heights, Illinois). All assays were performed in duplicate, and the assay sensitivity was 0.5 μg/ml. The intra-assay coefficient of variation was less than 5%. The results with hCS are expressed in ng/mg tissue.

Placental tissue was obtained in order to determine the tissue levels of hCG. Tissue was washed three times in McCoy's medium, frozen to −20° C and thawed three times to disrupt cells, and homogenized in a Thomas tissue homogenizer. The homogenate was centrifuged at 3,000 × g for 30 minutes and the supernatant was frozen at −20° C to await hCG analysis.

Statistical analysis was performed by means of Student's t test.

Results.

Spontaneous secretion of hCG. The mean concentration of hCG in seven tissues prior to incubation was

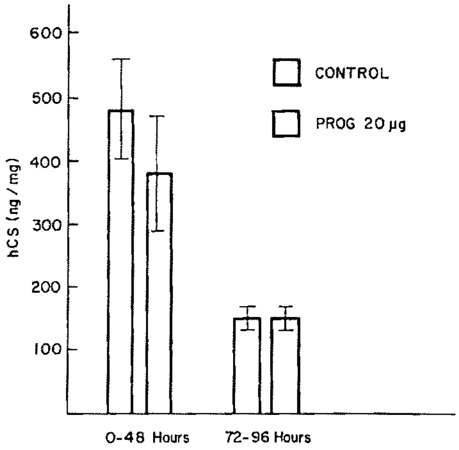

Fig. 4. Secretion of hCS (mean ± SEM) by placental explants cultured in the presence and absence of progesterone 20 μg/ml culture medium.

3.4 mIU/mg placenta, but the concentrations varied widely, with a range from 0.8 to 6.6 mIU/mg.

When values were expressed as the percent increase in hCG from baseline tissue concentrations, the pattern of hCG secretion under these conditions was similar for each placenta despite the wide range in initial tissue levels of hCG (Fig. 1.). Minimal secretion of hCG was observed for the first 48 hours, but a progressive and significant increase in hCG secretion occurred during the last 4 days of incubation. The first significant increase in hCG accumulation in the medium occurred between 48 and 72 hours of incubation, and a sixfold increase in hCG secretion was observed at 144 hours of incubation. Compared to tissue concentrations, the mean (± SEM) percent increase for seven placentas was 133.6 ± 17.7% at 48 hours, 213.9 ± 16.2% at 72 hours ($p < 0.05$), 378.7 ± 12.5% at 96 hours ($p < 0.001$), and 677.7± 101.7% at 144 hours ($p < 0.001$).

Effect of progestational steroids on hCG secretion. The secretion of hCG into the culture medium was markedly reduced in the presence of progesterone, 20 μg/ml (Fig. 1). Compared to control concentrations of hCG, little, if any, increase in hCG was observed in the P-treated cultures even after 144 hours of incubation ($p < 0.001$). Compared to initial tissue concentrations, hCG in the treated medium did not change significantly throughout the incubation period. This suppressive effect of P on hCG was consistent and highly significant for all tissues studied. Progesterone suppressed the secretion of hCG in a dose-response manner ($r = -0.8837$, $P < 0.1$). In the presence of P, 1 and 2 mcg/ml of culture medium, hCG concentrations did not differ from control values, but, as shown in Fig. 2, a significant decrease in the secretion of hCG was observed in the presence of progesterone 5 mcg ($P < 0.01$), progesterone 10 mcg ($P < 0.001$), and progesterone 20 mcg ($P < 0.001$). The suppression of hCG was also observed when progesterone was added to the culture medium at 96 hours ($P < 0.02$) after the increase in the secretion of hCG had begun (Fig. 2). When tissues were cultured in the presence of progesterone, a delayed increase in hCG was observed for the first 48 hours, similar to that seen in control cultures.

Placental tissue was also cultured in the presence of other steroids in order to eliminate a nonspecific steroid effect. Secretion of hCG did not significantly differ from control values when placental tissue was cultured in the presence of 20 mcg/ml of T, DHT, E_2, and E_3. The principal placental metabolite of progesterone, 20α-P, 20 μg/ml, significantly suppressed the secretion of hCG (27.2 ± 5.5 mIU/mg versus 6.9 ± 1.0 mIU/mg, $P < 0.01$) at 144 hours in a manner similar to that of progesterone. A significant increase in the concentration of hCG was caused by 17α-hydroxyprogesterone, 20 μg/ml, at 144 hours, compared to control values (8.9 ± 1.7 mIU/mg versus 29.4 ± 4.5 mIU/mg, $P < 0.01$); and a highly significant increase in the secretion of hCG was also observed in tissues cultured in the presence of cortisol (97.9 ± 18.2 mIU/mg, $P < 0.001$).

In an effort to eliminate the effects of endogenous steroid production, placental tissue was incubated in the presence of aminoglutethimide, which blocks side-chain cleavage and steroidogenesis. In the presence of 10^{-4} to 10^{-6}M concentrations of aminoglutethimide, hCG secretion did not significantly differ from control values. An agent which blocks 3β-ol-dehydrogenase activity, WIN-24540, in concentrations which effectively blocked progesterone formation (5 by 10^{-4}M), caused a slight decrease in the secretion of hCG after 96 hours of incubation (4.4 ± 0.3 mIU/mg versus 2.1 ± 0.9 mIU/mg, $P < 0.05$). Because of these findings, hCG secretion was studied in the presence of pregnenolone, 20 mcg/ml. Pregnenolone caused a significant reduction in hCG secretion (2.2 ± 0.4 mIU/mg) compared to control values (5.0 ± 1.0 mIU/mg, $P < 0.01$) and compared to values in tissues in the presence of WIN-24540 alone (3.7 ± 0.2 mIU/mg, $P < 0.01$). No significant difference in hCG secretion was observed between cultures treated with pregnenolone alone or with WIN-24540 and pregnenolone. Finally, no significant difference in hCG secretion was observed between tissues cultured in pregnenolone alone and progesterone alone.

In the presence of bu_2cAMP, a significant increase in the secretion of hCG was observed compared to control cultures for all time periods studied, even at 48 hours of incubation (Fig. 3). The augmented hCG response in the presence of bu_2cAMP ($P < 0.001$ at 144 hours) was significantly, but not completely, suppressed by progesterone 20 μg/ml ($P < 0.01$). The hCG concentrations at 48 hours in the presence of both bu_2cAMP and progesterone, 20 μg, were similar to those in the presence of bu_2cAMP alone, but the secretion of hCG was blunted at subsequent time periods in a manner similar to that in progesterone-treated cultures.

Effect of P on hCS secretion. In contrast to hCG, maximum secretion of hCS was observed during the first 48 hours of incubation. Control placental explants secreted 480 ± 39 ng/mg tissue during the first 48 hours compared to 150 ± 18 ng/mg between 72 and 96 hours of incubation (Fig. 4). No significant difference in hCS secretion was observed in placenta cultured in the presence of P, 20 μg/ml.

Comment

The pattern of spontaneous hCG secretion in the control cultures of these experiments is similar to results reported by other investigators.[8-10] The synthesis of hCS and hCG under these conditions has been confirmed by radioimmunoassay, immunoprecipitation, radioreceptor assay, and [^{3}H]-leucine incorporation.[6] Little, if any, hCG is secreted by placental explants in the first 48 hours of incubation. Golander and associates[8] found that hCG released into the medium during the first 24 to 48 hours was almost all preformed, since cyclohexamide had no effect on the hCG during this period of time, but did markedly suppress hCG synthesis after 48 hours of incubation. Presumably, the same influence which suppresses hCG synthesis and secretion in vivo continues to affect hCG synthesis for the first 48 hours in vitro. Thereafter, a linear increase in hCG secretion is observed for the next 96 hours. The length of the latent period is consistent with the time required for new protein synthesis.

The factors which control placental hormone synthesis and secretion are unknown. The human placenta was considered to be autonomous in its secretion of hormones until recently, when Khodr and Siler-Khodr[11] reported an increase in hCG in response to luteinizing hormone–releasing hormone (LHRH) which had been isolated from the placenta previously.[12] LHRH presumably stimulated the production of cAMP, also present in significant quantities in the placenta,[13] which increases hCG[14, 15] and aromatase activity[15] in vitro but has no effect on hCS secretion.[14] Although these observations could explain the increase in hCG early in gestation, the mechanisms responsible for the subsequent decline in the secretion of hCG remain unclear. The results of this in vitro study suggest that the synthesis of progesterone by the placenta may selectively suppress hCG secretion without affecting hCS secretion in vivo.

Large amounts of progesterone were used in these in vitro experiments, but these concentrations have been shown to be in the physiologic range for tissue levels. Concentrations of progesterone in retroplacental blood are 10 times those in maternal peripheral blood,[6] and concentrations in placental tissue vary from 2 to 10 μg per gram of tissue.[7] These levels are likely to be even greater in the isolated syncytiotrophoblast, the cell of origin for both progesterone and hCG. Concentrations of progesterone of 5 to 20 gm/ml of culture medium caused significant suppression of hCG secretion in this study. If uniform distribution of progesterone in the tissue and culture media is assumed, these concentrations are well within the physiologic range for placental tissue. Large concentrations of progesterone did not affect tissue viability because hCG secretion occurred normally after exposure of tissue to progesterone during the first 48 hours.

The suppression of hCG by progesterone is not a nonspecific steroid effect, because no suppression of hCG was observed even in the presence of pharmacologic amounts of C_{19} and C_{18} steroids. The response in the presence of other C_{21} steroids varied. Pregnenolone and 20α-dihydroprogesterone, the major placental metabolite of progesterone, suppressed hCG secretion, but 17α-hydroxyprogesterone and cortisol both increased hCG secretion under these conditions. The effects of both pregnenolone and 20α-dihydroprogesterone can be explained by interconversion to progesterone by the placenta.[17] However, the suppression of hCG by pregnenolone and WIN-24540, an inhibitor of 3β-ol-dehydrogenase activity, suggests that pregnenolone alone can also inhibit hCG synthesis. Aminoglutethimide, which blocks the synthesis of progesterone, was expected to cause an increase in the synthesis of hCG, but this was not observed. The lack of progesterone in this case does not necessarily enhance the secretion of hCG beyond that observed for control cultures.

The effects of 17-OHP and F on placental secretion of hCG differed from those of other steroids. A significant increase in hCG levels was observed in the presence of these agents. This effect could be mediated by displacement of progesterone from a serum corticosteroid-binding globulin in placental tissue, a similar placental steroid-binding protein, a specific progesterone receptor, or a specific glucocorticoid receptor. The mechanism by which 17-OHP and F exert their effects on hCG secretion is being investigated.

The mechanism by which progestational steroids or their metabolites decrease hCG secretion was not investigated in this study, but previous evidence would indicate a selective decrease in the rate of synthesis of the hormone. The mRNA concentration of alpha subunit of hCG is five to eight times greater in first-trimester placenta than in second- or third-trimester placenta,[3, 18, 19] and the mRNA for the beta subunit is nondetectable at term.[3] The tissue concentrations of the alpha subunit parallel plasma levels of hCG, and the rate-limiting factor in hCG synthesis is probably the synthesis of the beta subunit.[3] The biologic activity of progesterone is manifested by the induction or inhibition of protein synthesis. Previously established examples of inhibition by progesterone of protein synthesis are its inhibition of casein mRNA synthesis in the rat mammary gland[20] and its regulation of uterine excitability.[21] This hypothesis for progesterone action requires high placental concentrations of progesterone early in gestation in order to suppress hCG synthesis. Mean total concentrations of progesterone in maternal serum at the end of the first trimester are 41 ± 5 (±SEM) ng/ml, approximately one third of the amount found at term.[5] Fetal plasma concentrations of progesterone remain approximately the same (400 ng/ml) from 12 weeks' gestation until term,[4] and more fetal plasma progesterone is unbound to serum protein because of the low concentrations of corticosteroid-binding globulin in fetal blood.[22] Placental tissue concentrations of progesterone between 12 and 18 weeks' gestation (2.2 ± 2.2 μg/gm) are also similar to those found at term.[4] Thus, the concentrations of progesterone at the end of the first trimester may be sufficient to cause the suppression of hCG synthesis.

A small but consistent secondary rise in hCG in maternal serum[23] and placental tissue[24] during the last trimester of pregnancy has been reported. Boroditsky and associates[23] found that the levels of hCG increased significantly with advancing gestation in the serum of women bearing female fetuses, but no change in the concentrations of hCG were shown for women bearing male fetuses. Although no sex difference in maternal concentrations of progesterone were found,[23] male fetuses have higher umbilical artery levels of progesterone than do female fetuses.[25] These reports, in conjunction with the findings in the present study, suggest that the fetus may exert some control over the placental production of hCG.

In summary, under these in vitro conditions, progestational steroids, or their immediate metabolites, suppress the secretion of hCG. Increasing placental synthesis of progestin may be responsible for the decline in plasma hCG observed during human gestation. This efficient mechanism for the selective suppression of placental protein hormone synthesis may be equally important in the modulation of protein synthesis by other embryonic tissues.

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Subdermal estradiol pellets following hysterectomy and oophorectomy

Effect upon serum estrone, estradiol, luteinizing hormone, follicle-stimulating hormone, corticosteroid binding globulin–binding capacity, testosterone-estradiol binding globulin–binding capacity, lipids, and hot flushes

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Subdermal estradiol (E_2) pellets (25 mg) were inserted immediately after hysterectomy and oophorectomy in 22 menstruating women, ages 29 to 50 years. Serum samples were obtained daily for 7 days, weekly for 4 weeks, and at monthly intervals for 6 months. Although there was significant variation between patients, E_2 levels remained within the follicular phase range, averaging 50 to 70 pg/ml for 3 months, and then slowly declining to a mean of 37 pg/ml at 6 months, when new pellets were inserted. Over the entire study period, the E_2:estrone (E_1) ratio was greater than unity. Subdermal E_2 pellets limited the rise in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) after gonadectomy and the levels of LH and FSH 6 months after the insertion of E_2 pellets were significantly lower ($p < 0.01$) than in 20 postmenopausal women who had undergone oophorectomy and whose serum E_2 levels were less than 20 pg/ml. Serum corticosteroid binding globulin–binding capacity (CBG-BC) and serum testosterone–estradiol binding globulin–binding capacity (TeBG-BC) remained unchanged with E_2 pellets. Although high-density lipoprotein–cholesterol increased significantly ($p < 0.05$), low-density lipoprotein–cholesterol, total cholesterol, and triglycerides were unaffected, except for a rise in triglycerides in three older women with diabetes mellitus and hypertension. There were no complaints of severe hot flushes. Women who had vasomotor symptoms had mild or moderate flushes that occurred at 5 or 6 months after replacement of the pellets. Thus, E_2 pellets are an effective form of parenteral estrogen replacement therapy and offer both practical and theoretical advantages over other forms of estrogen replacement. (AM. J. OBSTET. GYNECOL. 138:714, 1980.)

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Supported in part by a grant from the Ford Foundation.

Presented at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980.

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NUMEROUS estrogen preparations and modes of administration have been developed in an effort to find which will relieve climacteric symptoms and prevent osteoporosis. Among the forms of therapy, subdermal implants of crystalline estradiol (E_2) have been used since 1938.[1-6] Both relief of vasomotor symptoms and the patient's preference over oral or parenteral estrogen therapy have been reported.[7] However, there is a paucity of data in regard to the levels of estrogen and gonadotropins after implantation of E_2 pellets. Hunter and associates[8] studied 23 patients for 15 months after

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the administration of one E_2 pellet containing 100 mg. Because the serum levels of E_2 exceeded those attained during the midfollicular phase of the cycle, it was apparent that only a lower dose could provide physiologic levels of E_2. The purpose of this study was to investigate the effects of the subcutaneous placement of one 25 mg E_2 pellet at the time of gonadectomy upon serum levels of estrogens, gonadotropins, lipids, and binding proteins and upon hot flushes, for a 6-month period.

Material and methods

The study group consisted of 22 women, ages 29 to 50 years, who underwent a hysterectomy and bilateral salpingo-oophorectomy because of severe salpingitis, leiomyomata uteri, or persistent menorrhagia or menometrorrhagia. Prior to operation, 15 women had ovulatory menstrual cycles and seven were anovulatory, but all had serum levels of follicle-stimulating hormone (FSH) or luteinizing hormone (LH) which were within the normal follicular phase range. All the patients elected to use E_2 pellets for replacement therapy. One 25 mg crystalline E_2-17β pellet (Progynon) was inserted subdermally with a trocar through a separate skin incision adjacent to the laparotomy incision after closure of the laparotomy incision.

Blood samples were obtained 1 day prior to operation and at 6 and 12 hours after insertion of the pellet and then daily at 9:00 A.M. for 7 days, weekly for the first month, and then monthly for 6 months. The serum samples obtained before and at 1, 2, 3, and 4 weeks and at 2, 3, 4, 5, and 6 months after implantation of the pellet were analyzed in all women. In addition, the blood drawn during the first week after insertion of the pellet was analyzed for E_2 in 10 women randomly selected from among the 22 patients. Serum lipids were analyzed in only 12 patients. LH and FSH were assayed in only 15 patients.

The results obtained from analyses of the sera of these women were compared to those obtained from a control group of 20 postmenopausal women, ages 50 to 60 years, who had undergone hysterectomy and bilateral salpingo-oophorectomy at least 2 years earlier. Although the ages of these women differed from those of our study patients, their weights were similar. Despite the fact that none of these control women was followed immediately after operation, there is ample evidence in the literature that hormonal levels are in the postmenopausal range 2 weeks after oophorectomy.[3] The samples of blood from these postmenopausal women were obtained after an overnight fast. None had taken any estrogen-containing compounds for at least 3 months. In addition, three of these 20 postmenopausal women volunteered to ingest 10 μg of ethinyl estradiol (EE_2, Estinyl) for 2 weeks. Twenty-microgram capsules of Estinyl were carefully divided in half and weighed on a microbalance to assure equal weights of each half. Venous blood for analysis was drawn prior to and at the end of the intake of EE_2. Blood was also obtained from another three of the 20 women before and after they had ingested conjugated estrogens (Premarin), 0.625 mg daily for 2 weeks.

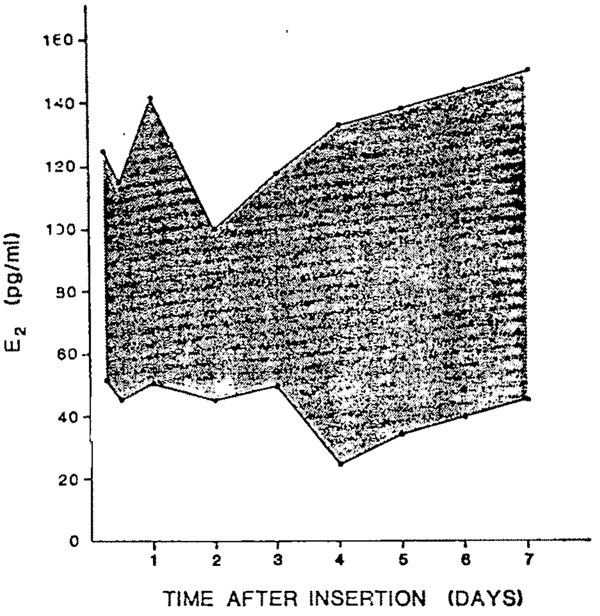

Fig. 1. The range of serum estradiol (E_2) concentrations during the initial 7 days after the insertion of one 25 mg E_2 pellet in 10 premenopausal women who underwent hysterectomy and oophorectomy.

In every instance, the blood was allowed to clot and the serum was separated and stored at −20° C for no longer than 3 months. Assays were carried out on these samples by means of procedures previously described for E_2 and E_1[10] and LH and FSH.[11] Determination of corticosteroid binding globulin–binding capacity (CBG-BC)[12] and testosterone-estradiol binding globulin–binding capacity (TeBG-BC) was carried out in our laboratory[13] with a modification of the method of Rosner[14] based on the binding of TeBG in plasma to tritiated dihydrotestosterone. Total cholesterol and total triglycerides were determined enzymatically with the use of a system Gilford 3500 computer-directed analyzer (Gilford Instruments, Oberland, Ohio). High-density lipoprotein(HDL)–cholesterol was measured enzymatically after precipitation with sodium phosphotungstate and magnesium chloride.[15] Low-density lipoprotein(LDL)–cholesterol was calculated.[16] All assays from each individual patient were performed at the

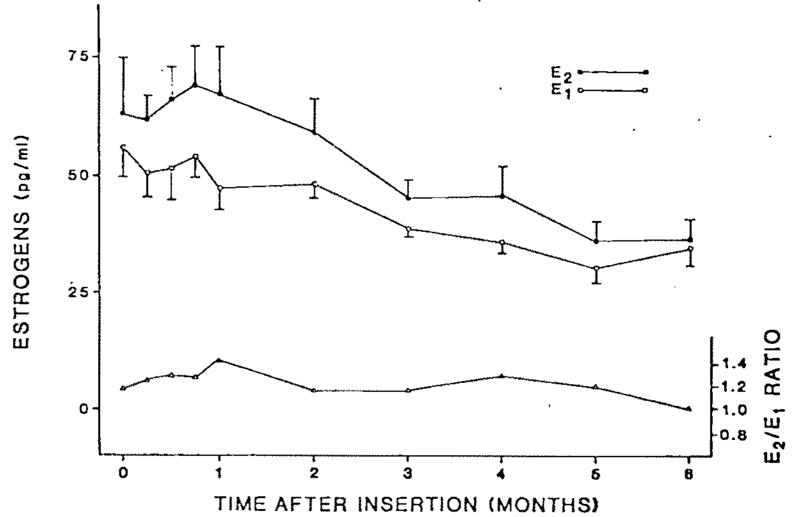

Fig. 2. Mean (±SE) serum estradiol (E_2) and estrone (E_1) levels and E_2/E_1 ratios in 22 women after oophorectomy and insertion of one 25 mg E_2 pellet.

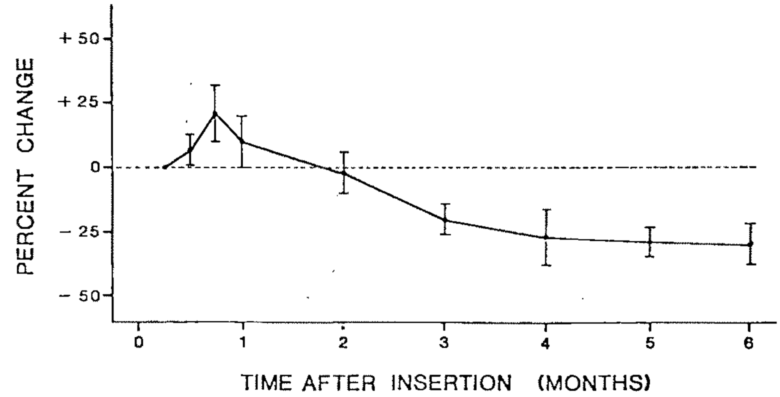

Fig. 3. Variation in serum levels of estradiol (E_2) after oophorectomy and insertion of a 25 mg E_2 pellet in 22 women. Values represent means (±SE) of each women's E_2 level expressed as percent of her serum E_2 level 7 days after insertion of the pellet. Values are expressed as percent change.

same time in order to decrease interassay variability.

No women received any medications chronically. Ten women received short-term, broad-spectrum antibiotic therapy in the first 7 to 10 days postoperatively. In this study, measurements on blood were carried out mainly after this first week. One woman took an oral hypoglycemic agent (tolbutamide) sporadically.

Information about the frequency and severity of hot flushes was obtained by oral questions at the time of each patient's visit and was characterized as mild, moderate, or severe. Occasional flushes which did not affect the patient's daily routine were defined as being mild. Moderate flushes were defined as those which occurred more often than twice per day and were troublesome but did not affect the patient's routine. Severe flushes were characterized as being frequent and affecting daily routines, so that the patient was led to seek medical assistance. Paired t tests were used to judge statistical significance.

Results

The range of E_2 levels during the first 7 days after insertion of the pellet in 10 women is indicated in Fig. 1. After oophorectomy and subdermal placement, the pellet released E_2 slowly. There was no initial "burst" effect, and serum E_2 levels remained below 150 pg/ml in the first 7 days after implantation.

It was found that the mean level of E_2 rose gradually and was highest 3 weeks after insertion of the pellet and then declined slowly thereafter (Fig. 2). Mean E_1 levels were relatively stable for 2 months and then declined gradually. Therefore, the ratio of E_2 to E_1 re-

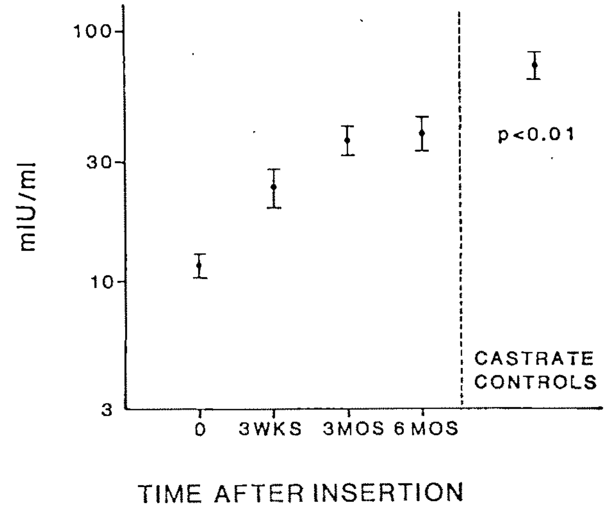

Fig. 4. Mean (±SE) serum LH levels (in mIU/ml) prior to and after oophorectomy and insertion of a 25 mg estradiol pellet in 15 premenopausal patients. The panel on the right depicts serum LH levels in 20 postmenopausal oophorectomized women not receiving estrogens.

mained greater than one throughout the study period. Estradiol levels in the postmenopausal control group were less than 20 pg/ml.

Estradiol levels varied widely among patients. Three weeks after implantation of the pellet, E_2 levels ranged from 30 to 122 pg/ml. Nevertheless, there was little variation in E_2 levels (expressed as percent change) within each patient during the 6 months (Fig. 3). When the individual values during 6 months were compared to the level attained 1 week after insertion of the pellet, the mean variation was found to be less than 30%. LH and FSH levels (in mIU/ml expressed as mean ± SEM) remained within the normal range for the first 3 weeks after implantation of the pellet and then rose progressively (Figs. 4 and 5). However, even 6 months after insertion of the pellet, LH and FSH levels were significantly lower than those of postmenopausal controls ($p < 0.01$). There was no significant change in the mean ± SEM CBG-BC in the patients with a pellet (Fig. 6). Two weeks of treatment with 0.625 mg of conjugated estrogens daily did not increase CBG-BC; however, 10 μg of ethinyl estradiol per day did cause a significant increase in CBG-BC ($p < 0.05$).

The mean ± SEM TeBG-BC also remained normal in the women who had a pellet, but both conjugated estrogens and ethinyl estradiol induced a significant elevation of TeBG-BC ($p < 0.05$) (Fig. 7).

HDL-cholesterol levels increased significantly ($p < 0.05$), whereas LDL-cholesterol and total cholesterol were not significantly altered (Fig. 8). For the patients as a group, total triglycerides were significantly increased ($p < 0.05$). However, this increase was due to a marked elevation (percent increase of 585, 414, 394) which occurred in three women who had both hypertension and diabetes mellitus. None of the subjects had severe hot flushes during the 6 months of treatment with the pellets (Table I). Five women never had a hot flush, whereas eight had mild hot flushes and nine had moderate flushes, primarily in the fifth and sixth months after insertion of the pellet.

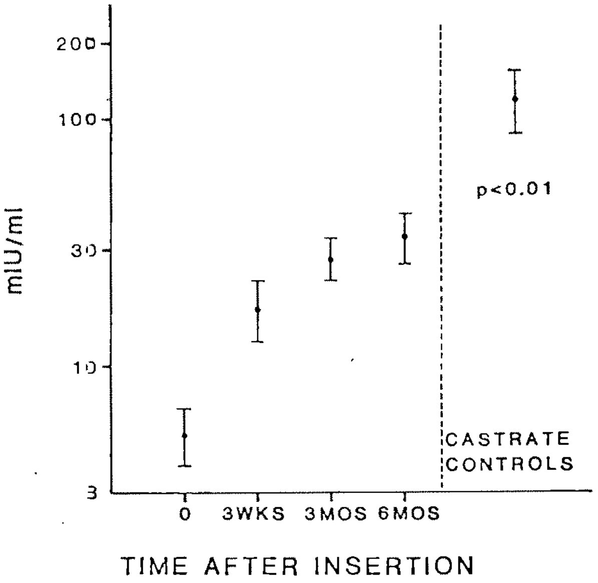

Fig. 5. Mean (±SE) serum FSH levels (in mIU/ml) prior to and after oophorectomy and the insertion of a 25 mg estradiol pellet in 15 postmenopausal women. The panel on the right depicts serum levels of FSH in 20 postmenopausal women not receiving estrogens.

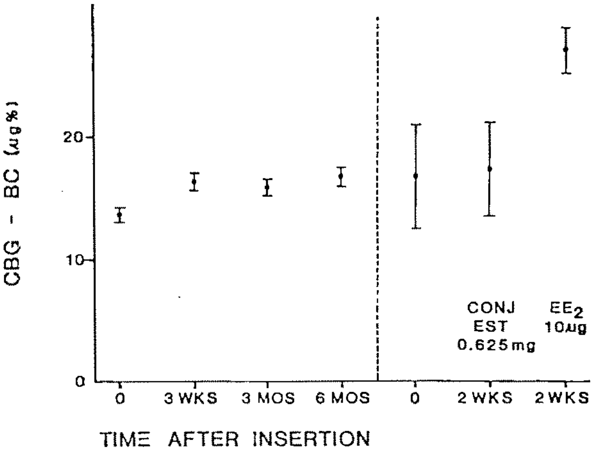

Fig. 6. Mean (±SE) CBG-BC, in μg%, prior to and after oophorectomy and the insertion of a 25 mg estradiol pellet in 22 women. The panel on the right shows the effect of 0.625 mg of Premarin and 10 μg of Estinyl (EE_2) on CBG-BC in six postmenopausal women.

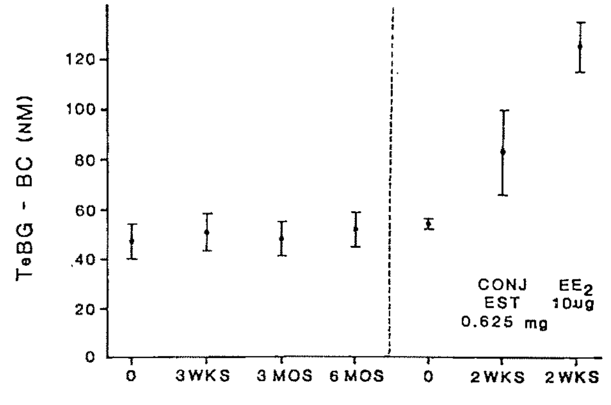

Fig. 7. Mean (±SE) TeBG-BC, in nM, prior to and after oophorectomy and insertion of a 25 mg estradiol pellet in 22 women. The panel on the right shows the effect of 0.625 mg of Premarin and 10 μg of Estinyl (EE_2) on TeBG-BC in six additional postmenopausal women.

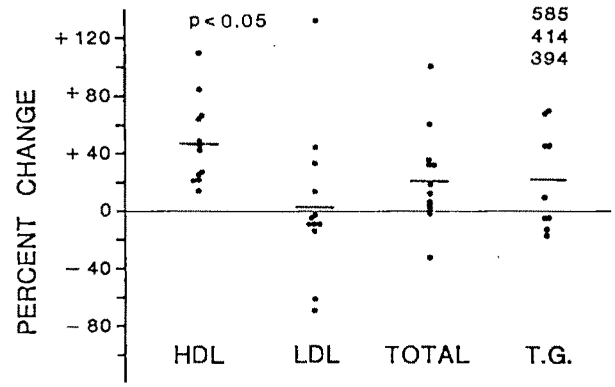

Fig. 8. Precent changes in serum lipids at 3 months compared to preoperative levels in 12 women undergoing oophorectomy and the insertion of a 25 mg estradiol pellet. Three women with diabetes mellitus and hypertension had markedly elevated serum triglycerides (394% to 585% change) and are depicted separately. *HDL,* high-density lipoprotein–cholesterol; *LDL,* low-density lipoprotein–cholesterol; *Total,* total cholesterol; *T.G.,* total triglycerides.

Table I. Complaint of hot flushes after oophorectomy and the insertion of a 25 mg estradiol pellet in 22 women

	Months					
Hot flushes	*1*	*2*	*3*	*4*	*5*	*6*
None	20	22	21	20	5	5
Mild	2	0	0	1	10	8
Moderate	0	0	1	1	7	9
Severe	0	0	0	0	0	0

Comment

This study describes the effect of continuous estrogen replacement therapy by means of a subdermal pellet containing 25 mg of crystalline E_2. This mode of therapy offers several advantages. Release of E_2 from crystalline pellets is rapid and fairly constant during the life span of the pellet. In contrast, oral administration of estrogen causes markedly elevated serum estrogen levels which are observed immediately and which are then followed by low levels. When E_2 is administered parenterally, it reaches all tissues without prior passage through the liver, so that the effective hepatic conversion of E_2 to E_1 is less than that which occurs after oral therapy. Thus, the E_2 to E_1 ratio remains greater than one as it occurs during normal ovarian function. Serum E_2 and E_1 levels remain within normal early and midfollicular phase ranges for at least 6 months.

Acceptance by the patient of this method was excellent. At the end of 6 months, only two women preferred not to continue therapy with the E_2 pellet, for personal reasons. Hot flushes were never severe and initially occurred 4 or more months after implantation of the pellet.

Only one 25-mg E_2 pellet was used in this study. Various authors have used two or more pellets at a time[8, 17]; however, in an earlier study, we found that one 25 mg pellet was sufficient to achieve adequate serum E_2 concentrations.[8]

Although E_2 levels after insertion of the pellet remained fairly constant within each patient, they varied widely among different subjects. This is at least partly related to the distribution of body fat. In this study, the more obese the woman, the lower were the total serum E_2 levels ($p < 0.05$). This effect may have been related, in part, to the sequestration of E_2 in fat and, in part, to the greater and altered peripheral conversion in obese women.[19]

Treatment with subdermal E_2 pellets results in a more protracted rise in serum LH and FSH after oophorectomy. Serum levels of LH and FSH remained within the normal premenopausal range for 3 weeks and then became elevated. However occasional individual values were still in the normal premenopausal range at 6 months. No correlation was found between individual levels of E_2 and gonadotropins in any one patient.

CBG is a hepatic protein whose binding capacity is known to correlate with increasing levels of estrogen.[20] In this study, E_2 pellets did not affect the levels of CBG-BC, in contrast to the significant increase produced by the ingestion of a small amount of ethinyl estradiol.

Serum TeBG-BC is a more sensitive indicator of the effects of estrogen upon hepatic proteins.[21] Although both the conjugated and synthetic oral estrogens increased the levels of TeBG-BC, the E_2 pellets did not alter the levels from baseline.

With regard to serum lipids, LDL-cholesterol and total cholesterol remained essentially unchanged. Total triglycerides increased in the patients as a group, but this was secondary to an effect upon three women. These greatly increased levels of triglycerides occurred in older women, ages 47, 48, and 50. All three had hypertension and either overt diabetes (one) or chemical diabetes mellitus (two). One of these women was receiving an oral hypoglycemic agent. In these three women, HDL, LDL, and total cholesterol were in the normal ranges, and triglyceride levels were only elevated at 3 months. The values at 3 months ranged from 347 to 677 mg/dl. Careful monitoring of such patients is needed if estrogen therapy is to be used.

In conclusion, the use of E_2 pellets is an effective, well-tolerated, and safe parenteral form of estrogen replacement therapy. It offers many practical and theoretical advantages over other forms of replacement therapy.

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Adult manifestation of congenital adrenal hyperplasia due to incomplete 21-hydroxylase deficiency mimicking polycystic ovarian disease

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This study was carried out to document the postpubertal presentation of congenital adrenal hyperplasia (CAH), to elaborate the diagnostic criteria for it, and to investigate family members of CAH patients. Serum 17-hydroxyprogesterone (17OHP) was measured in normal women and 25 hirsute oligomenorrheic patients, five of whom were shown to have CAH. These five CAH patients, as a group, had significantly elevated levels of 17OHP when compared to normal and hirsute women, although the other 20 hirsute oligomenorrheic women also had higher levels of 17OHP than the follicular phase control subjects. A single intravenous bolus of 0.25 mg of adrenocorticotropic hormone (ACTH) caused much larger increases in 17OHP in all five CAH patients than in the control and hirsute women. The five CAH patients had decreased cortisol but normal 11-deoxycortisol responses to ACTH, thus indicating 21-hydroxylase deficiency (21HD). Clinically, they were indistinguishable from women with polycystic ovarian disease (PCO) and had basal serum levels of androgens and urinary 17-ketosteroids which were similar to those found in 47 other women presenting with the complaint of hirsutism. However, the androstenedione levels and androstenedione/cortisol ratios in response to ACTH were significantly higher in the five CAH patients than in both the normal and hirsute women. Of seven family members tested, two fathers and one mother had an intermediate 17OHP response to ACTH, thus suggesting heterozygosity. Human lymphocyte antigen (HLA) typing on family members indicated that the inheritance of the disorder may be linked to B antigens. Two siblings of one of the CAH patients had normal 17OHP responses to ACTH and also had a different HLA-B complement. These data document the existence of adult manifestation of CAH, due to 21 HD. This disorder presents with androgen excess and oligomenorrhea or amenorrhea and mimicks PCO. The diagnosis of it hinges upon the post-ACTH rise in 17OHP, whereas the levels of serum androgens and urinary 17-ketosteroids may be inconclusive. (AM. J. OBSTET. GYNECOL. 138:720, 1980.)

CONGENITAL adrenal hyperplasia (CAH) in females is almost always recognized at birth, presenting as female pseudohermaphroditism with varying degrees of virilization. Some endocrinologists doubt that CAH may manifest itself for the first time after puberty. Among the various blocks in cortisol biosynthesis leading to this disease, 21-hydroxylase deficiency (21HD) is most frequently encountered and has been studied extensively.[1, 2] CAH is transmitted as an autosomal recessive disorder.[3] Heterozygous carriers of CAH are unaffected, although recent studies have led to the discovery that certain heterozygous family members have an abnormal steroid response to adrenocorticotropic hormone (ACTH).[4]

In genetic mapping of this disorder, several investigators found that 21HD is linked to human lymphocyte antigens (HLA) and, in particular, is associated with the B antigens.[5] This has led to the antepartum diagnosis of this disorder through amniocentesis and cell cultures.[6] In contrast to 21HD, 11-hydroxylase deficiency (11HD), the second most common enzymatic block causing CAH, does not appear to be associated

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This study was supported, in part, by Grant HD 05932 from the National Institutes of Health, United States Public Health Service, Bethesda, Maryland.

Sponsored by the Society for Gynecologic Investigation.

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with a definable carrier state nor to be linked genetically to HLA.[7]

It is still a matter of controversy (1) whether incomplete 21HD may manifest postpubertally with androgen excess, menstrual irregularities, and infertility, mimicking polycystic ovarian disease (PCO) and (2) whether this attenuated form of adult-onset CAH is, indeed, the same disorder as that recognized in the neonate. Some data, including the results of a study of HLA and other antigens, suggest that the latter are two separate disorders.[8] The diagnostic hallmark of CAH is an increase in circulating 17-hydroxyprogesterone (17OHP), which normally is metabolized by 21-hydroxylase to 11-deoxycortisol, the immediate precursor of cortisol. In 21HD, and in 11HD, the levels of 17OHP are significantly elevated in patients with CAH. However, mild but significant increases in 17OHP have also been noticed in some hirsute women.[9] Since 17OHP is a product of ovarian as well as adrenal steroid biosynthesis, and since the levels of 17OHP may be increased in women with PCO[10] some investigators have questioned the diagnosis of the so-called adult-onset CAH.[11]

The aims of the present study were (1) to document the existence of adult manifestation of CAH, (2) to elaborate the diagnostic criteria which differentiate this disorder from PCO, and (3) to investigate some patients' family members for subtle aberrations in cortisol biosynthesis and HLA mapping, in an effort to evaluate the genetic linkage of this disorder.

Material and methods

Except for normal female controls, all subjects in this study were patients referred to the Gynecologic Endocrinology/Infertility Clinic at the Los Angeles County/University of Southern California Medical Center, Women's Hospital, between July, 1978, and March, 1980. The testing was carried out as part of the patients' diagnostic work-up for complaints of androgen excess, menstrual aberrations, and/or infertility.

Thirty-four healthy, nonhirsute, and regularly menstruating women, 20 to 34 years old, who were using nonhormonal contraception, were recruited among family planning clinic patients to volunteer as controls. Fifteen of these 34 subjects were in the follicular phase (days 5 to 12) and 19 were in the luteal phase (days 14 to 25). Fifty-two hirsute patients were evaluated during the course of this study. Twenty-five of these patients who complained of hirsutism, oligomenorrhea, and infertility were selected for detailed hormonal studies as outlined below. Most of these 25 patients whose symptoms were consistent with PCO had elevated serum concentrations of luteinizing hormone (LH). Five of the 25 patients were found to have 21HD, as proved below. These five patients with adult manifestation of CAH were 18 to 22 years old and clinically indistinguishable from the other 20 hirsute and oligomenorrheic women.

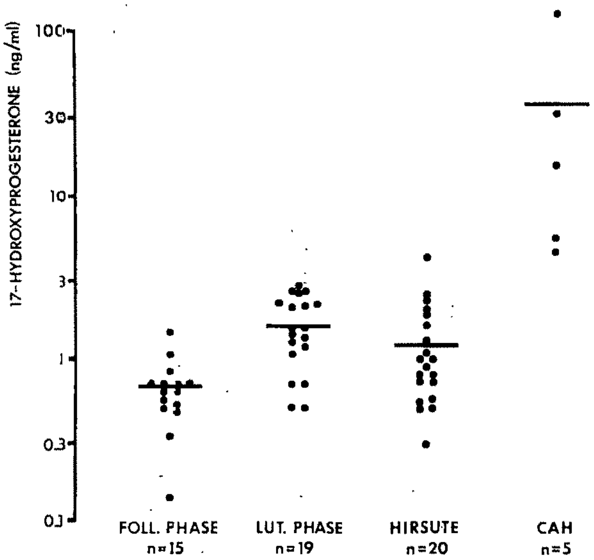

Fig. 1. Basal (8 to 10 A.M.) serum levels of 17OHP in 15 normal women in the follicular phase, 19 women in the luteal phase, 20 hirsute oligomenorrheic women, and five patients with CAH.

Venous blood was drawn between 8 and 10 A.M. Serum was separated by centrifugation within 1 hour and stored at − 15° C until times of analyses. Twenty-four-hour urines were collected and measured, and aliquots were frozen and stored at − 15° C until assayed for 17-ketosteroids (17KS) and creatinine. ACTH stimulation studies were carried out in seven normal female controls who were all in the luteal phase of their cycle, in five normal male controls, in five patients shown to have 21HD, in 10 of the remaining 20 hirsute and oligomenorrheic patients, and in seven family members of the 21HD patients. In this study, all subjects receiving ACTH were given 1 mg of dexamethasone orally at 11 P.M. the night before the ACTH test. At 8 A.M., a needle with a heparin lock was inserted into an arm vein, and blood was obtained 15 minutes prior to, at the time of, and 60, 120, and 180 minutes after 0.25 mg of intravenous (IV) synthetic ACTH (cosyntropin). In order to ascertain that a single IV bolus of 0.25 mg of ACTH was as effective as a continuous 3-hour IV infusion, 0.25 mg of ACTH dissolved in 30 ml of physiologic saline solution was infused intravenously in three female controls and three other normal women received a single IV bolus of 0.25 mg of ACTH. Blood was sampled prior to the administration of the ACTH and at 60, 120, and 180 minutes thereafter.

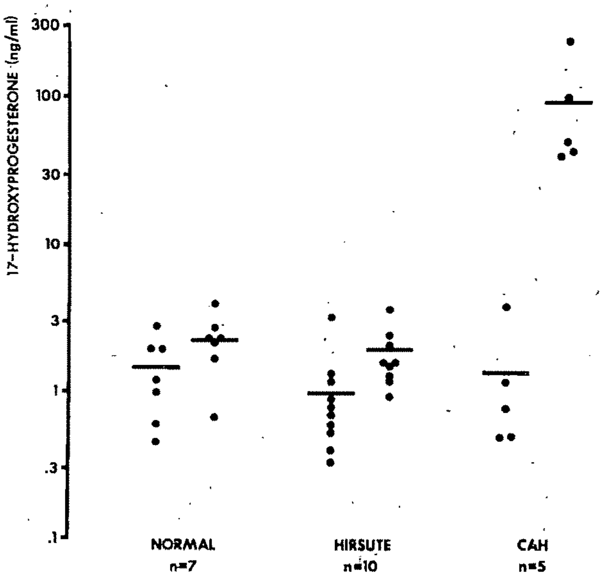

Fig. 2. Serum 17OHP before and 60 minutes after the IV administration of a single bolus of 0.25 mg of ACTH in seven normal female control and 10 hirsute oligomenorrheic women and in five patients with CAH.

Table I. Comparison of 170HP responses to ACTH in six control women*

Minutes	*IV infusion*	*IV bolus*
60	1.11 ± 0.50	1.21 ± 0.50
120	0.95 ± 0.40	1.01 ± 0.43
180	0.93 ± 0.56	1.20 ± 0.55

*Three women received an IV infusion of 0.25 mg of cosyntropin in saline solution over a 3-hour period. Another 3 women received a 0.25 mg IV bolus. Values are expressed as the mean (±SEM) increases in 170HP in ng/ml after 60, 120, and 180 minutes.

In four of the five CAH patients and seven of their family members, HLA typing was performed to determine antigens A, B, and C, by means of the standard two-stage National Institute of Health microcytotoxicity test.

Serum androstenedione (A), dehydroepiandrosterone sulfate (DHEA-S), 17-hydroxyprogesterone (17 OHP), and testosterone (T) was measured by radioimmunoassays (RIAs) as previously reported.[12–15] Serum cortisol (F) and 11-deoxycortisol (S) was determined by Endocrine Sciences, Tarzana, California, by specific RIAs. Urinary 17-ketosteroids (17KS) and creatinine were measured by Bio-Science Laboratories, Van Nuys, California. Student's t test was used to test for statistical significance when applicable.

Results

The 17OHP in normal and hirsute women, as well as in five patients shown below to have adult manifestation of CAH, is depicted in Fig. 1. The mean 17OHP level found in 20 hirsute oligomenorrheic women who did not have CAH was significantly higher than that observed in 15 nonhirsute women during their follicular phase ($p < 0.05$), but not significantly higher than the mean 17OHP level of 19 nonhirsute women during their luteal phase. The upper 95% confidence limits for 17OHP concentrations in nonhirsute women during the follicular and luteal phases was found to be 3.3 ng/ml. Only one of the 20 hirsute oligomenorrheic women had a 17OHP level (4.2 ng/ml) which was significantly higher than that of the nonhirsute control women. The five patients with suspected CAH had basal 17OHP concentrations ranging from 4.3 to 134 ng/ml, thus indicating that 17OHP levels in women with mild forms of CAH may overlap with the levels found in hirsute oligomenorrheic women. However, the response of 17OHP to exogenous ACTH separated the two groups of hirsute oligomenorrheic patients as shown below.

The ACTH stimulation test is more readily performed when ACTH is administered as a single IV bolus than as an IV infusion, and when samples of blood are drawn only at 0 and 60 minutes for 17OHP assay. In a pilot experiment in which six normal women received either a 3-hour IV infusion or a single IV bolus of 0.25 mg of ACTH, the results indicated that the response to the IV bolus does not differ from that to the 3-hour IV infusion (Table I). A maximum response is achieved by both methods within 60 minutes.

With the use of this convenient 1-hour ACTH stimulation test, the 17OHP response to ACTH in seven normal women, 10 of the 20 hirsute oligomenorrheic women who volunteered for extended evaluation, and the five CAH patients is depicted in Fig. 2. The ACTH-induced rise in 17OHP in the 10 hirsute oligomenorrheic women was indistinguishable from that found in the seven control women. The increase in 17OHP in the five CAH patients, however, was significantly greater than that observed in the seven control subjects and the 10 hirsute oligomenorrheic women, as well ($p < 0.005$). The 17OHP response to ACTH clearly separated these two groups of hirsute oligomenorrheic women. The 17OHP response to ACTH was also studied in five healthy men under identical conditions and was found to be indistinguishable from that observed in the seven female control subjects. The mean (±SEM) increases in the 17OHP concentrations in the five male and seven female controls were 1.0 ± 0.3 and 0.94 ± 0.24 ng/ml, respectively.

Basal serum cortisol concentrations were normal in all five CAH patients. Cortisol and 11-deoxycortisol was measured prior to and 60 minutes after ACTH stimulation in these five patients, as well as in the seven normal women. The cortisol response was significantly diminished ($p < 0.02$) in the five CAH patients, whereas the 11-deoxycortisol response was normal in four* (Fig. 3). Basally, all five women also had normal levels of 11-deoxycortisol. This indicates that these five women all had incomplete 21HD.

Serum A, DHEA-S, T, and 24-hour urinary 17KS, corrected for creatinine excretion were measured basally in the five CAH patients with incomplete 21HD, as well as the remaining 47 of the total 52 hirsute women with and without oligomenorrhea. The results shown in Fig. 4 indicate that A, DHEA-S, and T, as well as urinary 17KS levels in the five CAH patients were within the 95% confidence limits established for hirsute women, although some of the C-19 steroid ("androgen") levels in the five CAH patients were above those seen in normal women.

However, the A response to ACTH was significantly larger ($p < 0.05$) in four of the five CAH patients* than in the seven control and 10 hirsute oligomenorrheic women, between whom there was no difference in response (Fig. 5). The serum A/cortisol ratio after ACTH stimulation, combining the increased A and decreased serum cortisol response to ACTH in these five CAH patients, was markedly higher in the CAH patients than in the control and hirsute oligomenorrheic women (Fig. 5). Serum T levels rose by a mean (±SEM) of 0.46 ± 0.05, 0.44 ± 0.05, and 0.68 ± 0.15 ng/ml in response to ACTH stimulation in the seven control and 10 hirsute oligomenorrheic women, and in four of the five CAH patients,* respectively. The mean T response in the CAH patients was not significantly larger than that observed in the control and hirsute oligomenorrheic women ($0.1 > p > 0.05$). The five CAH patients received 0.5 or 0.75 mg of dexamethasone each day in divided doses. All resumed regular cycles, and the one patient who desired fertility conceived within 3 months and was delivered of an apparently normal female infant after 40 weeks' gestation.

Family members of two of the five CAH patients volunteered for further tests and, together with four of the five CAH patients, for HLA typing. The results of the HLA typing are listed in Table II. Two of the four patients were homozygous for the B_{14} antigen. The third patient was found to have one B_{14} and another undefined B antigen, whereas the fourth patient had one undefined B and a B_{w16} antigen. The results of the HLA typing performed on family members of two of the five CAH patients are shown in Fig. 6. Both fathers and mothers carried one B_{14} antigen, and both patients were homozygous for B_{14}. One patient was the only child. One of the two brothers and the sister of the other patient who volunteered to participate had no B_{14} antigens (Fig. 6). The 17OHP response to ACTH in these seven family members was determined and compared with that in the normal control and the hirsute oligomenorrheic women and the five CAH patients (Fig. 7). Both fathers and one mother had a 17OHP response which was significantly larger ($p < 0.05$) than that observed in five normal men, as well as in seven normal women and 10 hirsute oligomenorrheic patients.

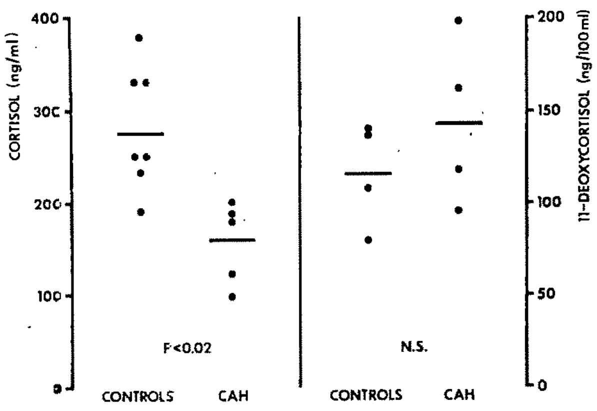

Fig. 3. Serum cortisol and deoxycortisol 60 minutes after the IV administration of a single bolus of 0.25 mg of ACTH in seven female control subjects and the five (four) patients with CAH.

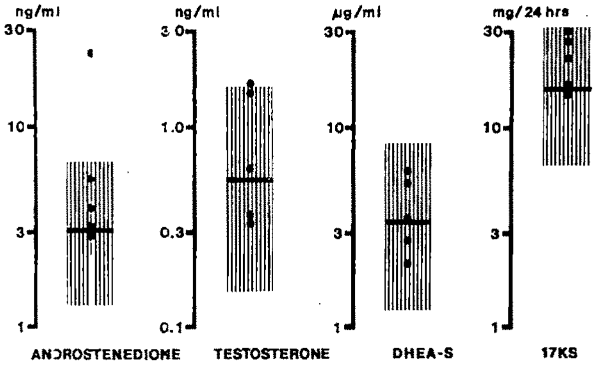

Fig. 4. Serum levels of androstenedione, testosterone, dehydroepiandrosterone sulfate *(DHEA-S)*, and urinary 17-ketosteroids *(17KS)* in the five patients with CAH (dots) as compared to the 95% confidence limits established for these steroids in 47 hirsute women (hatched bars).

Comment

The data indicate that CAH due to incomplete 21HD may manifest itself after puberty. These women present with complaints of hirsutism, menstrual irreg-

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*Serum available from the fifth patient was insufficient for analysis.

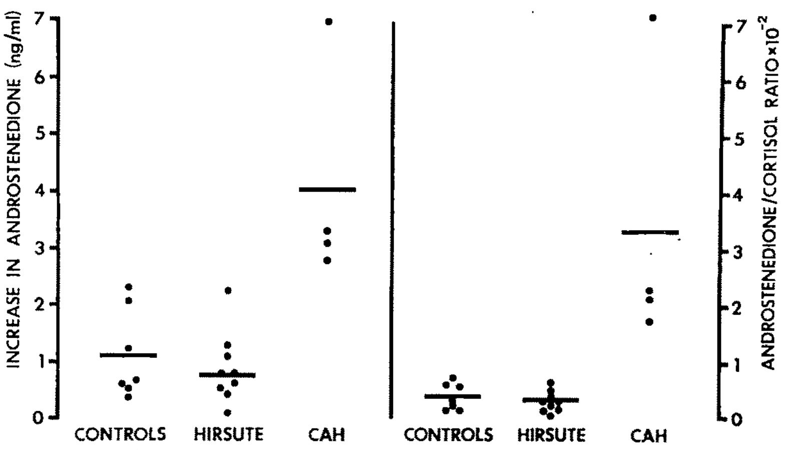


Fig. 5. Serum androstenedione and androstenedione/cortisol ratios 60 minutes after the IV administration of a single bolus of 0.25 mg of ACTH in seven female control and 10 hirsute oligomenorrheic women, and in four of the five patients with CAH.

Table II. HLA-ABA antigens in four women with CAH

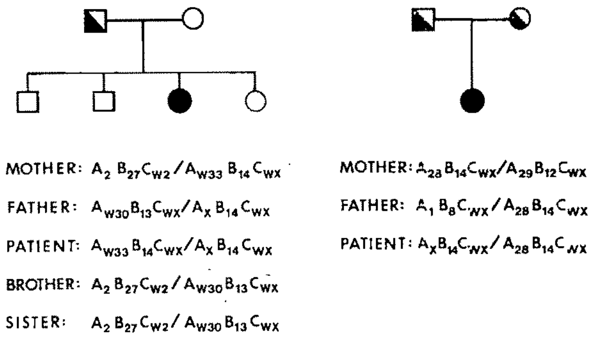
I. O.	$A_{w33}B_{14}C_{wx}/A_xB_{14}C_{wx}$
C. C.	$A_1B_{14}C_{w2}/A_xB_xC_{wx}$
J. B.	$A_{28}B_{14}C_{wx}/A_{28}B_{14}C_{wx}$
J. M.	$A_wB_{w13}C_{wx}/A_xB_xC_{wx}$

ularities, and infertility, and clinically are indistinguishable from other patients with mild-to-moderate androgen excess. In patients with adult manifestation of CAH, basal cortisol levels are normal, whereas basal A, DHEA-S, T, and urinary 17KS levels generally fall within the 95% confidence limits established for hirsute women. Serum levels of 17OHP are usually elevated above those of normal and hirsute oligomenorrheic women alike, but overlapping may occur. The most sensitive diagnostic test is the ACTH-induced rise in 17OHP, easily measured 1 hour after an IV bolus of 0.25 mg of ACTH after dexamethasone suppression. ACTH also induces an increased rise in A and a smaller rise in T in CAH patients. The ratio of A to cortisol after ACTH is significantly larger in all CAH patients, as compared to other hirsute oligomenorrheic women. These five patients had a decreased cortisol but normal 11-deoxycortisol response to ACTH, thus documenting incomplete 21HD. Although none of the five CAH patients had 11HD, this disorder may also cause adult manifestation of CAH presenting with androgen excess, oligomenorrhea or amenorrhea, and hypertension.

In vitro studies have suggested that 11HD may be induced by elevations in A, DHEA, and T levels.[16] The finding that our CAH patients had incomplete 21HD which was unaffected by androgens indicates that the rise in basal C-19 steroids and their increased response to ACTH are the result of, rather than the cause for, their mild defect in cortisol biosynthesis. Because A is secreted by the adrenals, and T results from the peripheral conversion of A, the basal levels of A and the ACTH-induced rises in A are larger than those observed for T in CAH patients. Thus, clinically, signs of androgen excess are similar in CAH patients and in other hirsute oligomenorrheic women who may have PCO.

The 17OHP response in the 10 hirsute oligomenorrheic women did not differ from that observed in seven control women tested during the luteal phase. This phase of the menstrual cycle was chosen because the suggestion has been made that the ACTH-induced 17OHP rise is smaller during the luteal than during the follicular phase, although the absolute 17OHP levels attained in response to ACTH have been higher in the luteal phase.[17] In this study, the rise in 17OHP was expressed in absolute serum concentrations. Our data which demonstrate no differences in the serum 17OHP response to ACTH between normal and hirsute oligomenorrheic women are in agreement with one report in the literature[17] and in disagreement with another.[18] Similarly, we did not observe a difference in the ACTH-induced rise in A between normal and hirsute oligomenorrheic women as reported by others.[18] These apparent discrepancies may be due to differences in the testing procedures.

It is well known that androgen excess accelerates linear growth and epiphyseal closure. Patients with CAH are ultimately shorter than expected based upon parental height. Patients who postpubertally are found to have CAH may also be shorter, and this may be an important clue. Three of our five CAH patients were



MOTHER: $A_2\ B_{27}C_{W2}/A_{W33}\ B_{14}C_{WX}$
FATHER: $A_{W30}B_{13}C_{WX}/A_X\ B_{14}C_{WX}$
PATIENT: $A_{W33}B_{14}C_{WX}/A_X\ B_{14}C_{WX}$
BROTHER: $A_2\ B_{27}C_{W2}/A_{W30}B_{13}C_{WX}$
SISTER: $A_2\ B_{27}C_{W2}/A_{W30}B_{13}C_{WX}$

MOTHER: $A_{28}B_{14}C_{WX}/A_{29}B_{12}C_{WX}$
FATHER: $A_1\ B_8C_{WX}/A_{28}B_{14}C_{WX}$
PATIENT: $A_XB_{14}C_{WX}/A_{28}B_{14}C_{WX}$

Fig. 6. Results of HLA typing of family members of two of the five patients with CAH. Black circles indicate the two affected women, and the half-black circle and squares indicate parents whose 17OHP response to ACTH is increased, thus suggesting heterozygosity.

less than 5 feet tall, whereas one was 5 feet 2 inches tall and another was 5 feet 5 inches tall. However, all three patients who were less than 5 feet tall were Mexican-Americans and within the height range of that ethnic group.

Recent studies have dealt with the aspects of heterozygosity in CAH and strengthened the suggestion that this disorder is genetically linked and similar to the condition seen in children. Our study confirms that a "heterozygous" ACTH-induced 17OHP response may be observed in some family members. Nevertheless, our understanding of the expression of this disorder in heterozygotes is incomplete, since in one of the two families tested, the mother did not exhibit an increased 17OHP response, as might be expected if this condition is an autosomal recessive disorder. However, it has been shown that the heterozygous response is not absolute[4] and is more variable in women.[17] The postpubertal expression of mild CAH due to incomplete 21HD may be determined by other factors, as well. Events such as adrenarche with increases in the production of steroid hormones may trigger the manifestation of this disorder.

HLA typing has added to our understanding of the genetic linkage in this disorder.[5] It is generally agreed that the gene responsible for 21HD is somehow associated with the B antigens. Nevertheless, to date, there is no particular B antigen which is characteristic of this disorder. The monitoring of HLA B antigens has been used mainly within families in order to elucidate the genetic transmission of 21HD. For these reasons and because of the wide variation of HLA genotypes which exist, it was considered to be unnecessary to do more extensive HLA typing on women without biochemical evidence for their disorder. In the two families tested for HLA, both parents passed on a B_{14} antigen to their affected children, who were two of our five CAH patients. In one of the two families, a sister and a brother who had a normal 17OHP response to ACTH had no B_{14} antigen. The third of the four CAH patients tested for HLA had only one documented B_{14} antigen, whereas the second B antigen was undetermined. The results obtained in the fourth CAH patient in whom no B_{14} antigen was found are explained by the assumption that incomplete 21HD may be linked to B antigens other than B_{14}, as stated earlier. The exact mode of inheritance of this disorder, the association of the gene with certain cellular antigens, and the control of the expression of this disorder remain enigmatic and warrant further investigation.

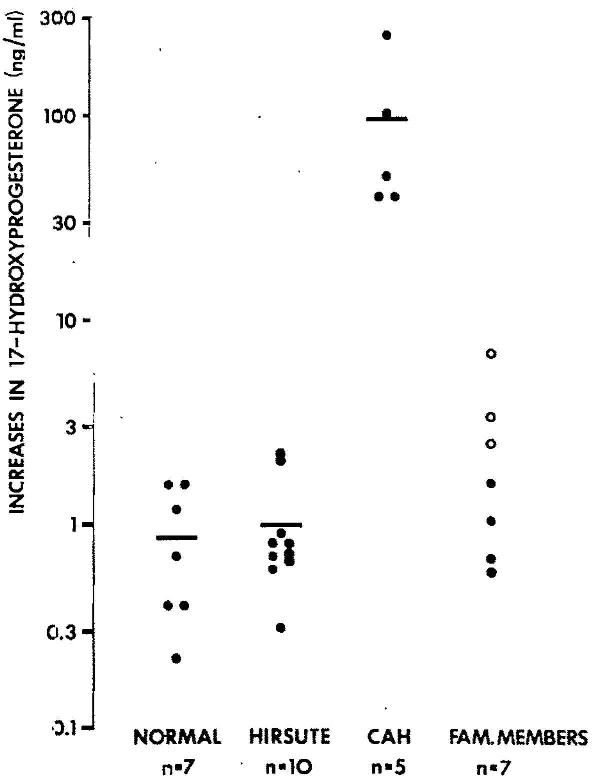


Fig. 7. Increases in serum 17OHP 60 minutes after the IV administration of a single bolus of 0.25 mg of ACTH in seven normal female control subjects, 10 hirsute oligomenorrheic women, and the five patients with CAH and seven of their family members.

In conclusion, adult manifestation of CAH does exist. Clinically, this disorder mimicks PCO, and the diagnosis of it hinges upon the 17OHP response to ACTH. This disorder is readily corrected with 0.5 to 0.75 mg of dexamethasone daily, given in divided doses. Family members of CAH patients may have an abnormal 17OHP response to ACTH, thus suggesting a "heterozygous" or carrier state. HLA typing and testing of the 17OHP response to ACTH in their spouses may be of help in the genetic counseling of women with the disorder, and HLA typing of amniotic fluid cell cultures may aid in the antenatal diagnosis of CAH.

We are indebted to Mr. Dan Ford and the staff at the Blood Bank Laboratory of the Los Angeles County/University of Southern California Medical Center for HLA typing, to Mrs. Helen R. Haase, M.Sc., for skillful technical assistance, and Ms. Lisa Rodriguez, R.N., for her efforts in dealing with patients and volunteers in our Gynecologic Endocrinology/Infertility Clinic.

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Indications: For the treatment of symptomatic trichomoniasis in females and males when the presence of the trichomonad has been confirmed by appropriate laboratory procedures (wet smears and/or cultures). Also for the treatment of asymptomatic females when the organism is associated with endocervicitis, cervicitis, or cervical erosion. *T. vaginalis* infection is a venereal disease and therefore, asymptomatic sexual partners of treated patients should be treated simultaneously if the organism is present. The decision to treat an asymptomatic male partner who has a negative culture, or one in whom no culture has been attempted, is an individual one. In any event, the consort should be treated with Flagyl in cases of reinfection. Flagyl is also indicated for acute intestinal amebiasis (amebic dysentery) and for amebic liver abscess.

Contraindications: Evidence or history of blood dyscrasia, active organic disease of the CNS, the first trimester of pregnancy and a hypersensitivity to metronidazole.

Warnings: Flagyl should not be used in the first trimester of pregnancy (see *Contraindications*). Use with discretion during the second and third trimesters of pregnancy and restrict to those pregnant patients not cured by topical measures. Flagyl (metronidazole) is secreted in the breast milk of nursing mothers. It is not known whether this can be injurious to the newborn. If used during lactation, an alternative method of feeding may be elected.

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Most prominent among the effects in the mouse was the promotion of pulmonary tumorigenesis. This has been observed in all five reported studies in that species, including one study in which the animals were dosed on an intermittent schedule (administration during every fourth week only). One of the mouse studies revealed increases in the incidence of malignant lymphomas as well as pulmonary neoplasms associated with lifetime feeding of the drug. All these effects are statistically significant.

In two long-term toxicity studies in the rat there was a statistically significant increase in the incidence of various neoplasms, particularly mammary tumors, among female rats on metronidazole over that noted in the control groups.

Two lifetime tumorigenicity studies in hamsters have been reported to be negative.

Metronidazole has been reported to potentiate the anticoagulant effect of coumarin and warfarin resulting in a prolongation of prothrombin time. This possible drug interaction should be considered when Flagyl is prescribed for patients on this type of anticoagulant therapy.

Precautions: Mild leukopenia has been reported during Flagyl (metronidazole) use; total and differential leukocyte counts are recommended before and after treatment with the drug, especially if a second course is necessary. Flagyl may interfere with certain chemical analyses for SGOT. Avoid alcoholic beverages during Flagyl therapy because abdominal cramps, nausea, vomiting, headaches and flushing may occur. Discontinue Flagyl promptly if abnormal neurologic signs occur. Exacerbation of candidiasis may occur. In amebic liver abscess, aspirate pus during metronidazole therapy.

Adverse Reactions: Nausea, headache, anorexia, vomiting, diarrhea, epigastric distress, abdominal cramping, constipation, a metallic, sharp and unpleasant taste, furry tongue, glossitis and stomatitis possibly associated with a sudden overgrowth of *Candida*, exacerbation of vaginal candidiasis, an occasional reversible moderate leukopenia, dizziness, vertigo, incoordination, ataxia, convulsive seizures, peripheral neuropathy, fleeting joint pains, confusion, irritability, depression, weakness, insomnia, mild erythematous eruptions, urticaria, flushing, nasal congestion, dryness of the mouth, vagina or vulva, pruritus, dysuria, cystitis, a sense of pelvic pressure, dyspareunia, fever, polyuria, incontinence, decrease of libido, proctitis, pyuria and darkened urine have occurred in patients receiving the drug. Patients receiving Flagyl may experience abdominal distress, nausea, vomiting, flushing or headache if alcoholic beverages are consumed. The taste of alcoholic beverages may also be modified. Flattening of the T-wave may be seen in ECG tracings.

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in two parts, part 1
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American Journal of OBSTETRICS AND GYNECOLOGY

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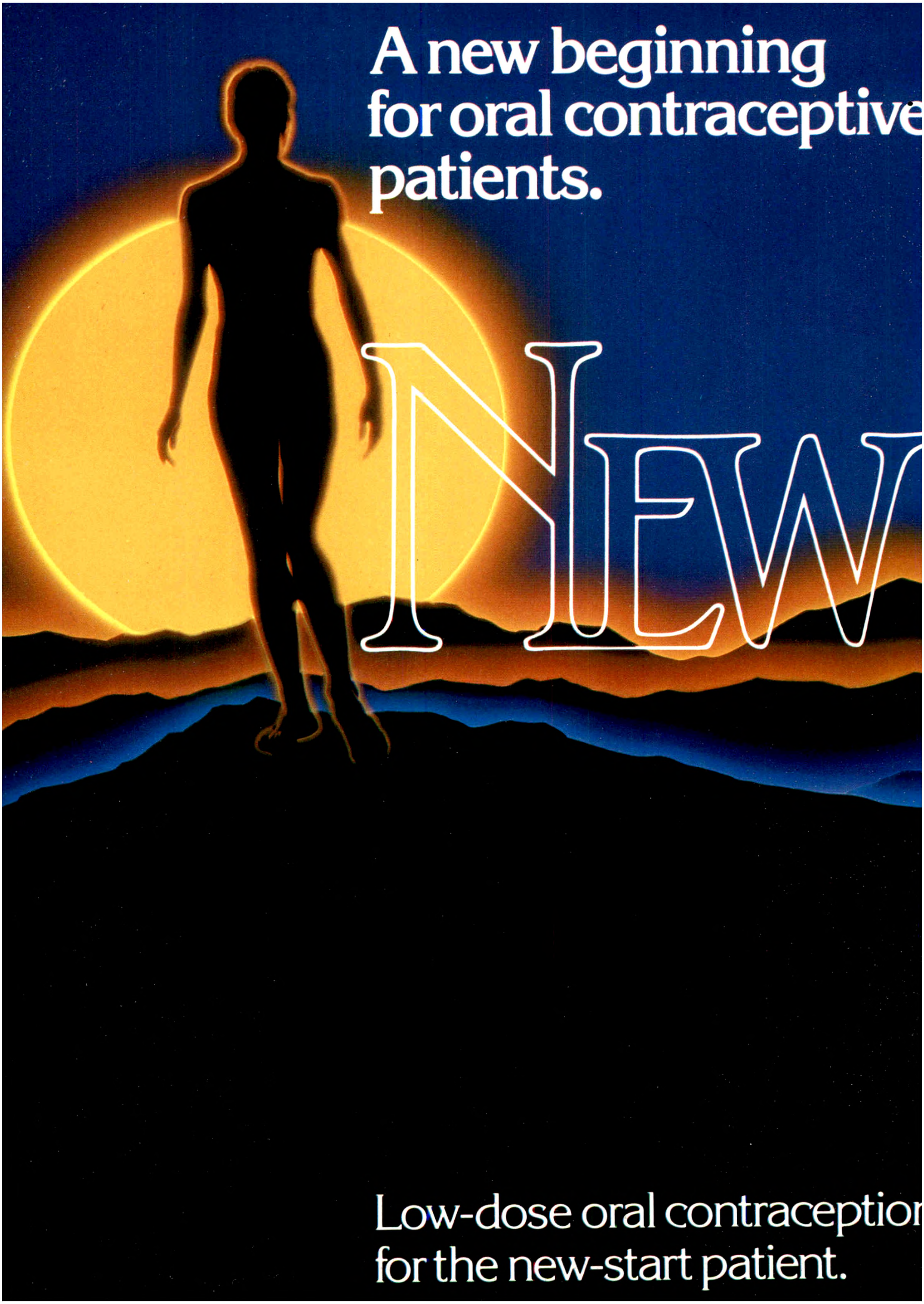
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WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from O.C. use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use O.C.s should be strongly advised not to smoke.

The use of O.C.s is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing O.C.s should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of O.C.s is well established. One British study demonstrated an increased relative risk for fatal venous thromboembolism; two British and three American studies demonstrated an increased relative risk for non-fatal venous thromboembolism. These studies estimate that users of O.C.s are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke, which had not been shown in prior British studies. In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the relative risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the relative risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers. A British long-term, follow-up study reported in 1976 a highly significant association between O.C. use and stroke. Another British long-term, follow-up study had suggested such an association in 1974, but the number of cases was too small to estimate the risk. MYOCARDIAL INFARCTION. An increased relative risk of myocardial infarction associated with the use of O.C.s has been reported, confirming a previously suspected association. One study conducted in the United Kingdom found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an O.C. user or not. O.C.s, however, were found to be an additional risk factor. In terms of relative risk, it has been estimated that O.C. users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. O.C. users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke, and about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the number of cigarettes smoked is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. (The estimates are based on British vital statistics which show acute myocardial infarction death rates 2- to 3-times less than in the U.S. for women in these age groups; consequently, actual U.S. death rates could be higher.) The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in O.C.s. Preparations containing 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been supported by a study in the United States. Careful epidemiological studies to determine the degree of thromboembolic risk associated with progestogen-only O.C.s have not been performed. Cases of thromboembolic disease have been reported in women using these products, and they should not be presumed to be free of excess risk. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASE. A large British prospective study estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of O.C.s according to age, smoking habits, and duration of use. The overall excess death rate annually from circulatory diseases for O.C. users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). The risk is concentrated in older women, in those with a long duration of use, and in cigarette smokers, and may persist after discontinuation of O.C. use. It was not possible, however, to examine the interrelationships of age, smoking, and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of O.C.s) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The findings of this analysis are shown in Table 1 below. The study concluded that the mortality associated with all methods of birth control is low and below that of childbirth, with the exception of O.C.s in women over 40 who smoke. (The rates given for pill only/smokers for each age group are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates given would be about double; for "light" smokers (less than 15 cigarettes a day), about 50 percent.) The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The study also concluded that O.C. users who smoke, especially those over the age of 30, have a greater mortality risk than O.C. users who do not smoke.

Table 1. *The annual number of deaths associated with control of fertility and no control per 100,000 nonsterile women by regimen of control and age of women are:*

	15-19	20-24	25-29	30-34	35-39	40-44
No method	5.6	6.1	7.4	13.9	20.8	22.6
Abortion only	1.2	1.6	1.8	1.7	1.9	1.2
Pill only —nonsmokers	1.3	1.4	1.4	2.2	4.5	7.1
Pill only —smokers	1.5	1.6	1.6	10.8	13.4	58.9
IUDs only	0.9	1.0	1.2	1.4	2.0	1.9
Traditional contraception only	1.1	1.6	2.0	3.6	5.0	4.2
Traditional contraception and abortion	0.2	0.2	0.3	0.3	0.3	0.2

The risk of thromboembolic and thrombotic disease associated with O.C.s increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclampic toxemia and especially by cigarette smoking. Based on

risk of death from circulatory disorders associated with the use of O.C.s:

Table 2. *Smoking habits and other predisposing conditions—risk associated with use of O.C.s*

Age	Below 30	30-39	40+
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (No predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A—Use associated with very high risk.
B—Use associated with high risk.
C—Use associated with moderate risk.
D—Use associated with low risk.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four- to six-fold increased risk of post-surgery thromboembolic complications has been reported in O.C. users. If feasible, O.C.s should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. Data also suggest that the presence of varicose veins substantially increases the risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. *Ocular Lesions:* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of O.C.s. Discontinue O.C. medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of certain adenomas, benign or malignant, such as those of the breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently contained in O.C.s, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies have reported an increased relative risk (3.1 to 13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women who took estrogen replacement medication to relieve menopausal symptoms. One publication reported on the first 30 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on O.C.s. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time O.C.s were first given, polycystic ovaries) nearly all occurred in women who had used a sequential O.C. These products are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only O.C.s, although individual cases have been reported. Several studies have shown no increased risk of breast cancer to women taking O.C.s or estrogens. In one study, however, while no overall increased risk of breast cancer was noted in women treated with O.C.s, a greater risk was suggested for the subgroups of O.C. users with documented benign breast disease and for long-term (2-4 years) users. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. A reduced occurrence of benign breast tumors in users of O.C.s has been well documented. In a prospective study of women with cervical dysplasia, there was an increase in severity and of conversion to cancer *in situ* in O.C. users compared with nonusers. This became statistically significant after 3 to 4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to be predictive of progression after prolonged exposure. There have been other reports of microglandular hyperplasia of the cervix in users of O.C.s. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with O.C.s. Close clinical surveillance of all women taking O.C.s is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with O.C. use. This has been reported in short-term as well as long-term users of O.C.s. One study reported that use of O.C.s with high hormonal potency and age over 30 years may further increase a woman's risk of hepatocellular adenoma. Two studies relate risk with duration of use, the risk being much greater after 4 or more years of use. Although it is an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may clearly show a focal defect. Hepatic arteriography may be a useful procedure in diagnosing primary liver neoplasm. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that O.C.s further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use O.C.s instead of other methods of contraception. Furthermore, a high percentage of women exposed to diethylstilbestrol (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported following use of sex hormones, including O.C.s, in pregnancy. In one case-control study it was estimated that there was a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (O.C.s, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including O.C.s, during early pregnancy occurred at a rate of 18.2 per 1,000 births, compared to 7.8 per 1,000 for children not so exposed *in utero*. These results are statistically significant. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing O.C.s. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping O.C.s is unknown. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period (or after 45 days from the last menstrual period if progestogen-only O.C.s are used) and further use of O.C.s should be withheld until pregnancy has been ruled out. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue O.C.s with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy. 6. *Gall Bladder Disease:* Studies report an increased risk of gall bladder disease in users of O.C.s or estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In another study an increased risk was apparent between 6 and 12 months of use. 7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on O.C.s. For this reason, prediabetic and diabetic patients should be carefully observed while receiving O.C.s. An increase in triglycerides and total phospholipids has been observed in patients receiving O.C.s. The clinical significance of this finding is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with O.C. use. In some women, hypertension may occur within a few months of beginning O.C. use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two and a half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in O.C. users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, a history of toxemia or elevated blood pressure during pregnancy, a familial tendency to hypertension or its consequences, or a history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevation of blood pressure when given O.C.s and, therefore, should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, the clinical implications of elevations should not be ignored and close surveillance is indicated, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after discontinuation of the O.C. 9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of O.C.s and evaluation of the cause. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses are frequent reasons for patients discontinuing O.C.s. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an O.C. with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of O.C.s. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased prevalence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing O.C.s in comparison with those discontinuing other methods of contraception. The impairment appears to be independent of the duration of use of the preparations. While the impairment diminishes with time, there is still an appreciable difference in the results in nulliparous women for the O.C. and non-O.C. groups 30 months after discontinuation of birth control. For parous women the difference is no longer apparent 30 months after cessation of contraception. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in progestogen-only O.C. failures, the ratio of ectopic to intrauterine pregnancies is higher than in women who are not receiving O.C.s, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* O.C.s given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in O.C.s has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast fed child have not been determined. If feasible. the use of O.C.s should be deferred until the infant has been weaned. **Precautions** *GENERAL* 1. A complete medical and family history should be taken prior to the initiation of O.C.s. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, O.C.s should not be prescribed for longer than 1 year without another physical examination being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking O.C.s should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. 4. O.C.s may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving O.C. therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. O.C. users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is unknown. 8. Serum folate levels may be depressed by O.C. therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping O.C.s, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of O.C. therapy when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing O.C.s. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. *DRUG INTERACTIONS* O.C.s may be rendered less effective by virtue of drug interaction with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, and antimigraine preparations. O.C.'s may alter the effectiveness of other types of drugs, such as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., quanethidine), vitamins and hypoglycemic agents. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reations has been associated with the use of O.C.s (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving O.C.s and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, change in corneal curvature (steepening) change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution in lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors. The follwing adverse reactions have been reported in users of O.C.s, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function. **Information for the Patient** (See Patient Package Insert)

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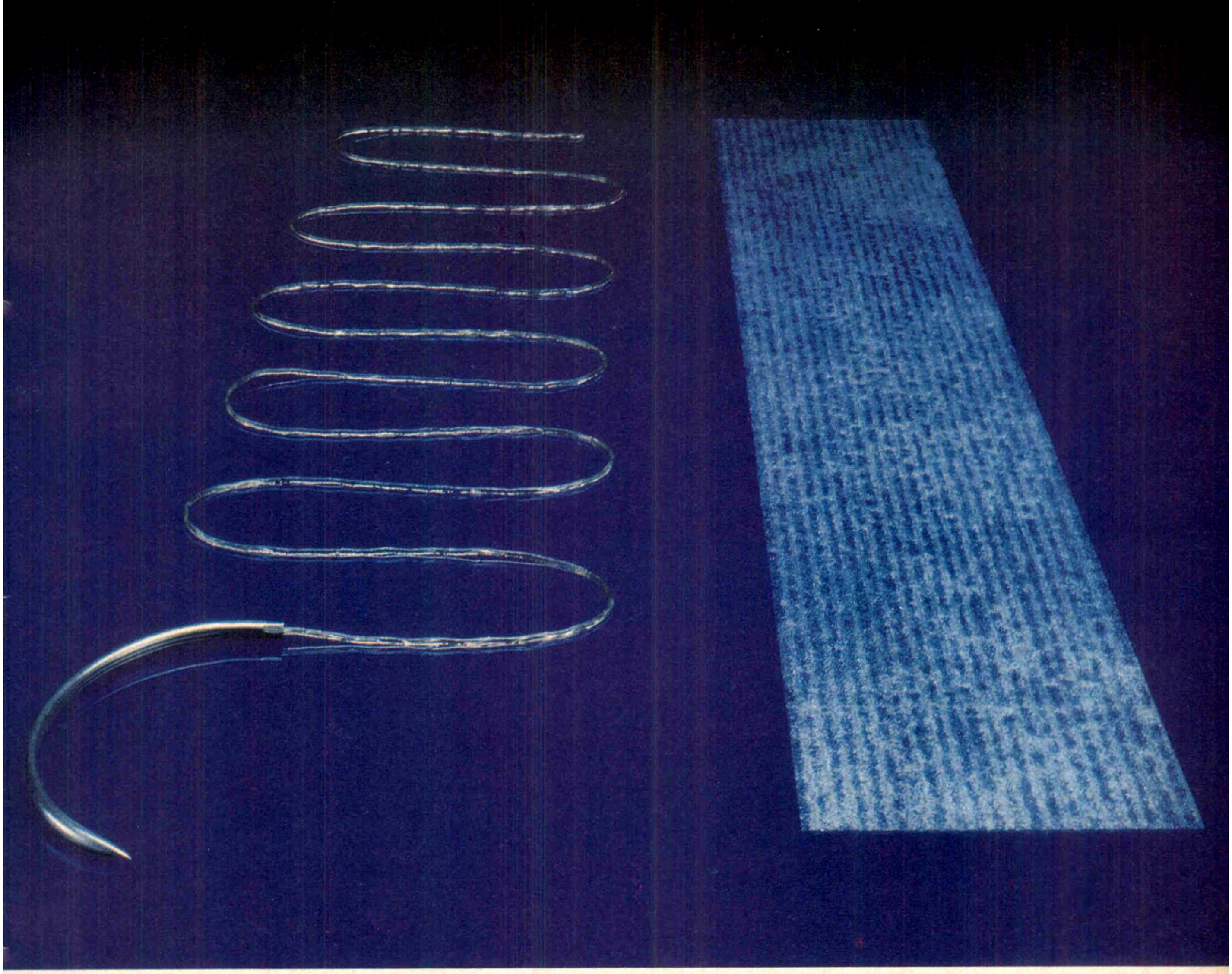

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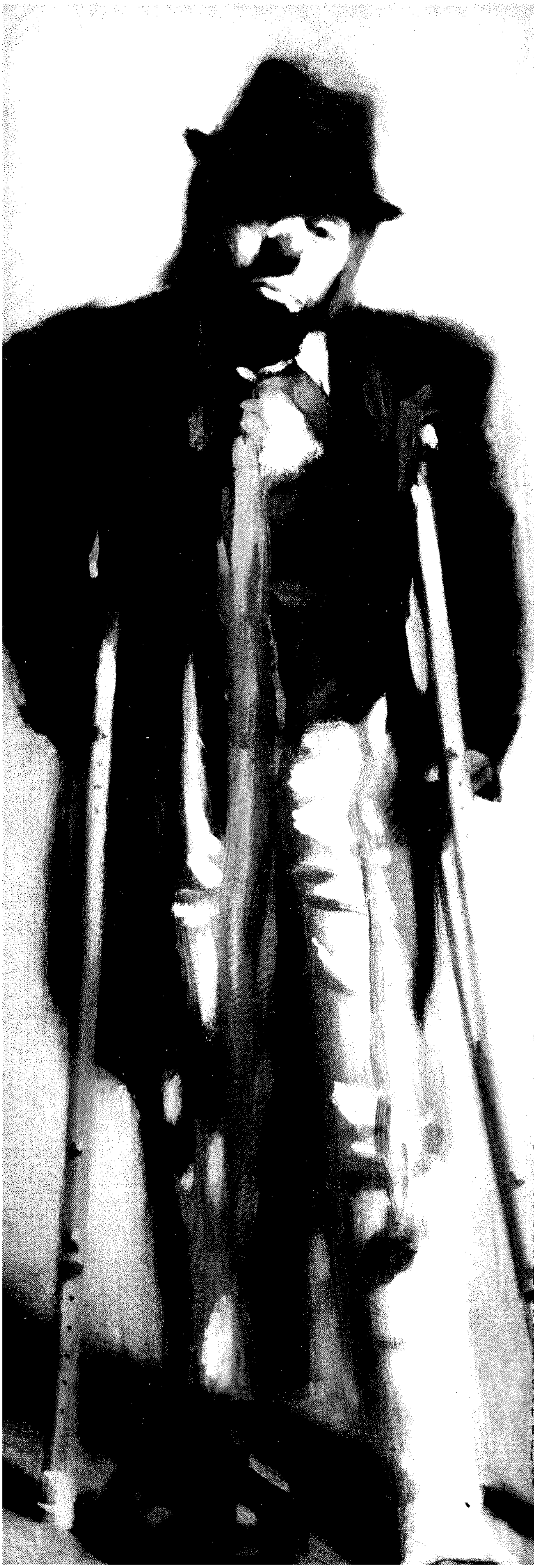

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WARNINGS:

Drug dependence: Empirin with Codeine can produce drug dependence of the morphine type and, therefore, has the potential for being abused. Psychic dependence, physical dependence, and tolerance may develop upon repeated administration of this drug and it should be prescribed and administered with the same degree of caution appropriate to the use of other oral, narcotic-containing medications. Like other narcotic-containing medications, the drug is subject to the Federal Controlled Substances Act.

Use in ambulatory patients: Empirin with Codeine may impair the mental and/or physical abilities required for the performance of potentially hazardous tasks such as driving a car or operating machinery. The patient using this drug should be cautioned accordingly.

Interaction with other central nervous system (CNS) depressants: Patients receiving other narcotic analgesics, general anesthetics, phenothiazines, other tranquilizers, sedative-hypnotics, or other CNS depressants (including alcohol) concomitantly with Empirin with Codeine may exhibit an additive CNS depression. When such combined therapy is contemplated, the dose of one or both agents should be reduced.

Use in pregnancy: Safe use in pregnancy has not been established relative to possible adverse effects on fetal development. Therefore, Empirin with Codeine should not be used in pregnant women unless, in the judgment of the physician, the potential benefits outweigh the possible hazards.

PRECAUTIONS:

Head injury and increased intracranial pressure: The respiratory depressant effects of narcotics and their capacity to elevate cerebrospinal fluid pressure may be markedly exaggerated in the presence of head injury, other intracranial lesions or a pre-existing increase in intracranial pressure. Furthermore, narcotics produce adverse reactions which may obscure the clinical course of patients with head injuries.

Acute abdominal conditions: The administration of Empirin with Codeine or other narcotics may obscure the diagnosis or clinical course in patients with acute abdominal conditions.

Allergic: Precautions should be taken in administering salicylates to persons with known allergies: patients with nasal polyps are more likely to be hypersensitive to aspirin.

Special risk patients: Empirin with Codeine should be given with caution to certain patients such as the elderly or debilitated, and those with severe impairment of hepatic or renal function, hypothyroidism, Addison's disease, prostatic hypertrophy or urethral stricture, peptic ulcer, or coagulation disorders.

ADVERSE REACTIONS: The most frequently observed adverse reactions to codeine include light-headedness, dizziness, sedation, nausea and vomiting. These effects seem to be more prominent in ambulatory than in nonambulatory patients and some of these adverse reactions may be alleviated if the patient lies down. Other adverse reactions include euphoria, dysphoria, constipation, and pruritus.

The most frequently observed reactions to aspirin include headache, vertigo, ringing in the ears, mental confusion, drowsiness, sweating, thirst, nausea, and vomiting. Occasional patients experience gastric irritation and bleeding with aspirin. Some patients are unable to take salicylates without developing nausea and vomiting. Hypersensitivity may be manifested by a skin rash or even an anaphylactic reaction. With these exceptions, most of the side effects occur after repeated administration of large doses.

DOSAGE AND ADMINISTRATION: Dosage should be adjusted according to the severity of the pain and the response of the patient. It may occasionally be necessary to exceed the usual dosage recommended below in cases of more severe pain or in those patients who have become tolerant to the analgesic effect of narcotics. Empirin with Codeine is given orally. The usual adult dose for Empirin with Codeine No. 2 and No. 3 is one or two tablets every four hours as required. The usual adult dose for Empirin with Codeine No. 4 is one tablet every four hours as required.

DRUG INTERACTIONS: The CNS depressant effects of Empirin with Codeine may be additive with that of other CNS depressants. See WARNINGS.

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Vol. 138, No. 7, Part 1, December 1, 1980. The American Journal of Obstetrics and Gynecology is published semimonthly by The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63141. POSTMASTER: Send address changes to above address.

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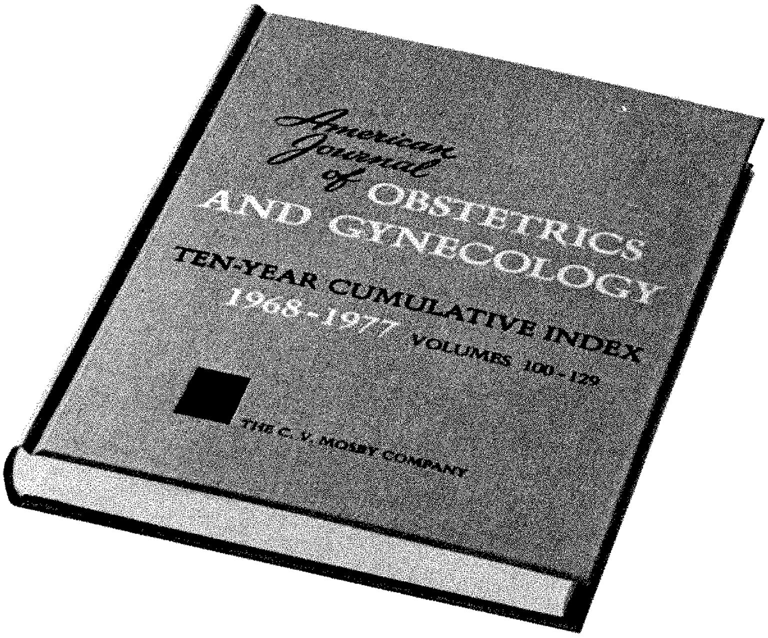
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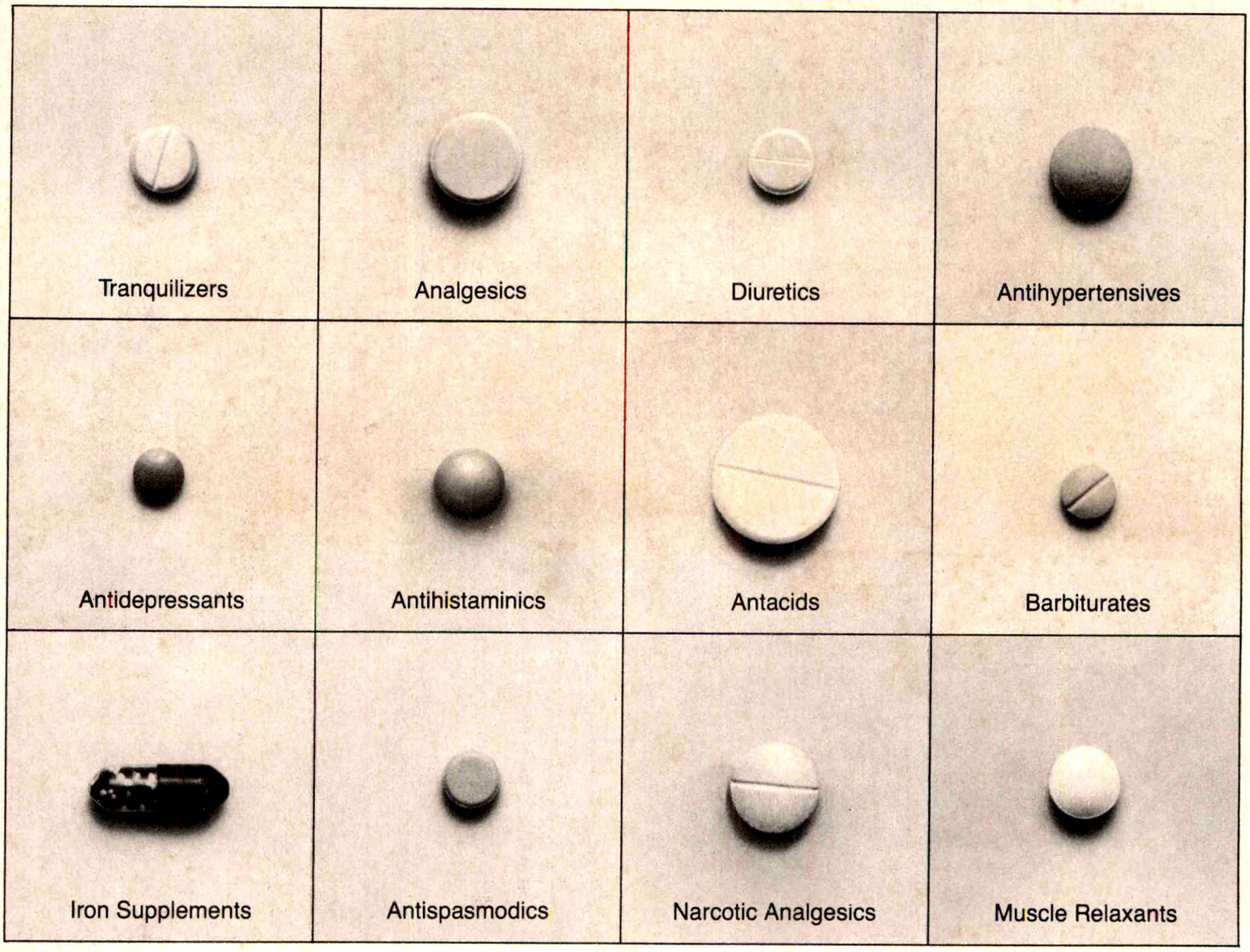
Many of the drugs you prescribe may also cause constipation



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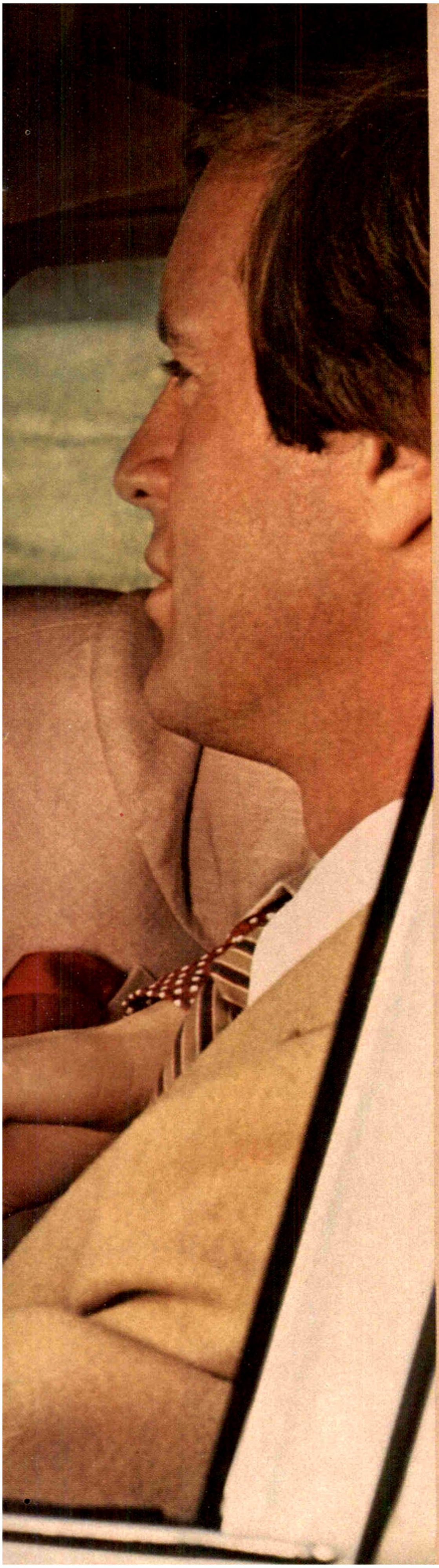

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Brief Summary of Prescribing Information

LOESTRIN® 1.5/30

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DESCRIPTION

Loestrin 1.5/30 Products are progestogen-estrogen combinations.

INDICATION AND USAGE

Loestrin 1.5/30 Products are indicated for the prevention of pregnancy in women who elect to use oral contraceptives.

In clinical trials with Loestrin 1.5/30 involving 17.139 therapy cycles, there was a pregnancy rate of 0.49 per 100 woman years.

Dose-related risk of thromboembolism from oral contraceptives: Studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. It is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance.

CONTRAINDICATIONS

1. Thrombophlebitis or thromboembolic disorders
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders
3. Cerebral vascular or coronary artery disease
4. Known or suspected carcinoma of the breast
5. Known or suspected estrogen-dependent neoplasia
6. Undiagnosed abnormal genital bleeding
7. Known or suspected pregnancy (See No. 5 under WARNINGS.)

WARNINGS

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
>
> The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, and hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well-established. Studies have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic.

Cerebrovascular disorders: In a collaborative study in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 4.0 to 9.5 times greater

Myocardial infarction: An increased risk of myocardial infarction associated with oral contraceptives has been reported confirming a previously suspected association. These studies found that the greater the number of underlying risk factors (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) for coronary artery disease, the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor.

It has been estimated that users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are smokers have about a fivefold increased risk of fatal infarction compared to users who do not smoke, but about a tenfold to twelvefold increased risk compared to nonusers who do not smoke. The amount of smoking is also an important factor.

Risk of dose: In an analysis of data, British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; however, the quantity of estrogen may not be the sole factor involved.

Estimate of excess mortality from circulatory diseases: The risk of diseases of the circulatory system is concentrated in older women, in those with a long duration of use, and in cigarette smokers.

A study of available data from a variety of sources concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke

The risk of thromboembolic and thrombotic diseases associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders. Should any occur or be suspected, the drug should be discontinued immediately.

A fourfold to sixfold increased risk of postsurgery thromboembolic complications has been reported in users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

2. *Ocular lesions:* Neuro-ocular lesions, such as optic neuritis or retinal thrombosis, have been associated with the use of oral contraceptives. Discontinue the oral contraceptive if there is unexplained sudden or gradual, partial, or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions.

3. *Carcinoma:* Long-term continuous administration of estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver

In humans, an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women has been reported. However, there is no evidence suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives.

Studies found no evidence of increase in breast cancer in women taking oral contraceptives; however, an excess risk in users with documented benign breast disease was reported

There is no confirmed evidence of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of users is, nevertheless, essential. In cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer, or who have breast nodules, fibrocystic disease, or abnormal mammograms, should be monitored with particular care.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with oral contraceptives. Because hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage, they should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock.

A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Usage in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* During early pregnancy, female sex hormones may seriously damage the offspring.

An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of oral contraceptives in pregnancy

There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives.

Pregnancy should be ruled out before continuing an oral contraceptive in any patient who has missed two consecutive menstrual periods. If the patient has not adhered to the schedule, the possibility of pregnancy should be considered at the time of the first missed period, and oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed.

Women who discontinue oral contraceptives with the intent of becoming pregnant should use an alternate form of contraception for a period of time before attempting to conceive.

Administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives.

7. *Carbohydrate and Lipid Metabolic Effects:* Because decreased glucose tolerance has been observed in a significant percentage of patients, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives.

An increase in triglycerides and total phospholipids has been observed.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving oral contraceptives. The prevalence in users increases with longer exposure. Age is also strongly correlated with development of hypertension. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure

9. *Headache:* Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, nonfunctional causes should be borne in mind. In undiagnosed abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy.

Women with a past history of oligomenorrhea or secondary amenorrhea, or young women without regular cycles should be advised that they may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast Feeding:* Oral contraceptives may interfere with lactation. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs.

PRECAUTIONS

1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another examination.

2. Preexisting uterine leiomyomata may increase in size.

3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

4. Oral contraceptives may cause fluid retention and should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice. If jaundice develops, the medication should be discontinued.

6. Steroid hormones may be poorly metabolized and should be administered with caution in patients with impaired liver function.

7. Users may have disturbances in normal tryptophan metabolism, which may result in a relative pyridoxine deficiency.

8. Serum folate levels may be depressed.

9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted

10. Certain endocrine and liver function tests and blood components may be affected.

(a) Increased sulfobromophthalein retention. (b) Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. (c) Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone. (d) Decreased pregnanediol excretion. (e) Reduced response to metyrapone test.

Drug interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin and an association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and ampicillin.

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with oral contraceptives.

Thrombophlebitis; Pulmonary embolism; Coronary thrombosis; Cerebral thrombosis; Cerebral hemorrhage; Hypertension; Gallbladder disease; Benign hepatomas; Congenital anomalies

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed. Mesenteric thrombosis, Neuro-ocular lesions, eg, retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms, breakthrough bleeding, spotting; change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma; breast changes; change in weight; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately post partum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature, intolerance to contact lenses.

The following adverse reactions have been reported and the association has been neither confirmed nor refuted

Premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis, porphyria.

Special Notes on Administration

Loestrin $\boxed{21}$ 1.5/30 (1.5 mg norethindrone acetate and 30 mcg ethinyl estradiol tablets, USP)—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after discontinuing medication

Loestrin $\boxed{\text{Fe}}$ 1.5/30—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after the brown ferrous fumarate tablets have been started.

Because of the relatively low estrogenic content, Loestrin 1.5/30 is not a good cyclic regulator. There are patients whose inherent hormone balance will require larger amounts of estrogen than that contained in Loestrin 1.5/30 to achieve cyclic regularity. These patients experience altered bleeding patterns, which do not conform to treatment schedules.

The physician should be alert to the fact that the irregular bleeding patterns could mask bleeding from organic cause, and appropriate diagnostic measures should be taken if the bleeding persists or continues after changing to a higher estrogen-content product.

After several months, bleeding may be reduced to a virtual absence; reduced flow may be a result of medication and not indicative of pregnancy.

HOW SUPPLIED

Loestrin $\boxed{\text{Fe}}$ 1.5/30 is available in compacts each containing 21 green tablets and 7 brown tablets. Each green tablet contains 1.5 mg of norethindrone acetate and 30 mcg of ethinyl estradiol. Each brown tablet contains 75 mg of ferrous fumarate. Available in packages of five compacts and packages of five refills.

Loestrin $\boxed{21}$ 1.5/30 is available in compacts each containing 21 tablets. Each green tablet contains 1.5 mg of norethindrone acetate and 30 mcg of ethinyl estradiol. Available in packages of five compacts and packages of five refills.

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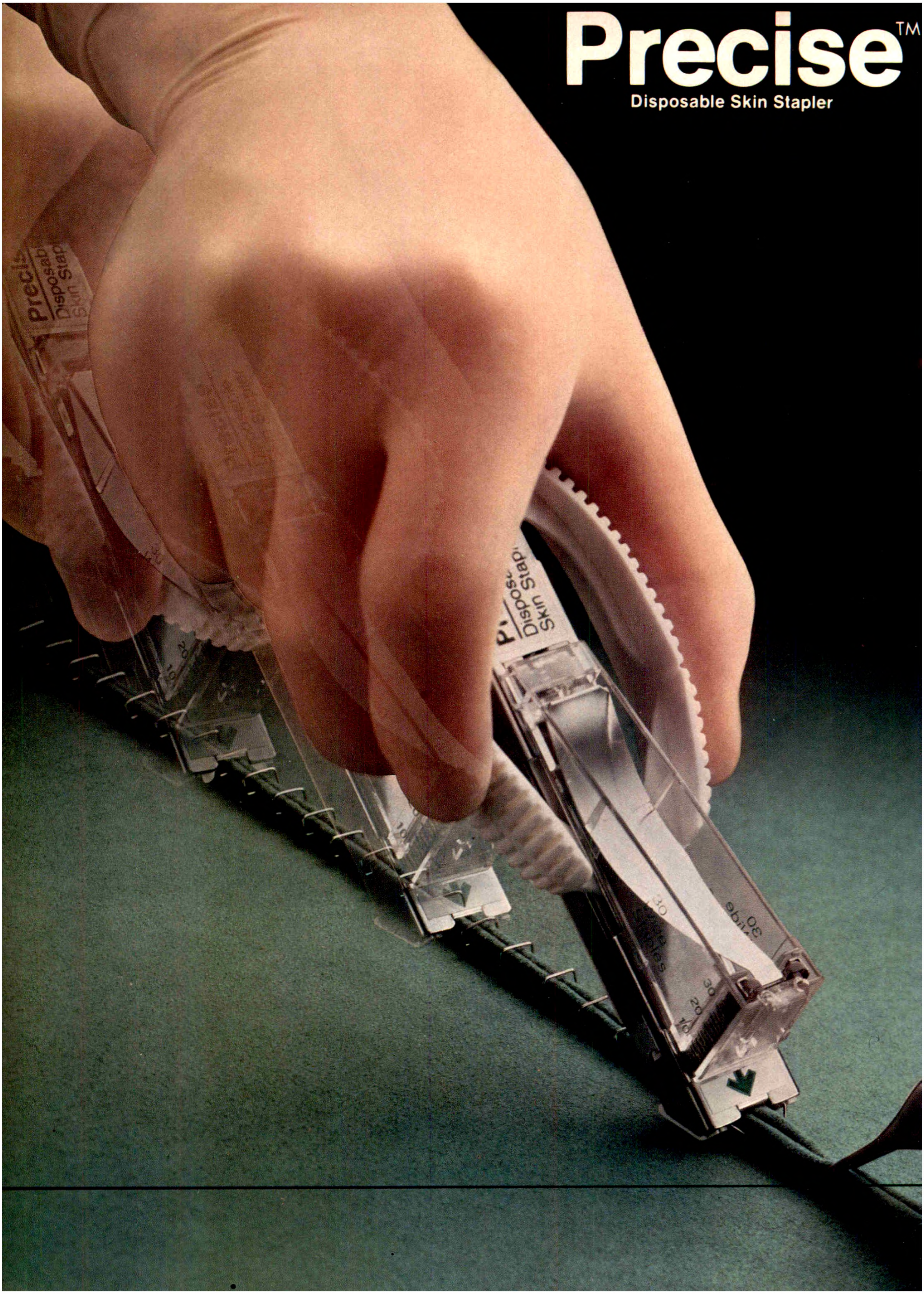
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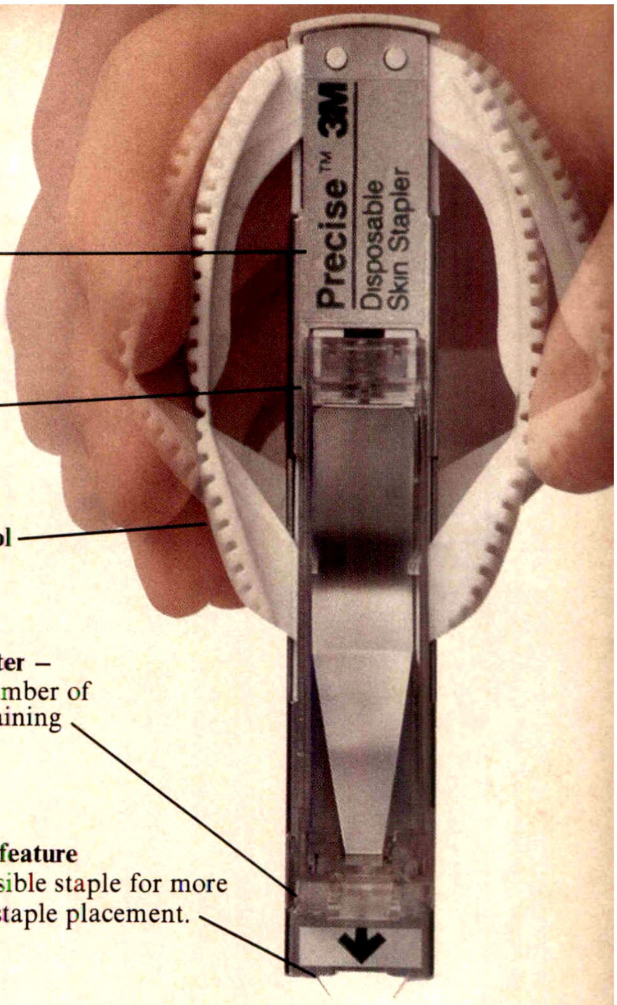

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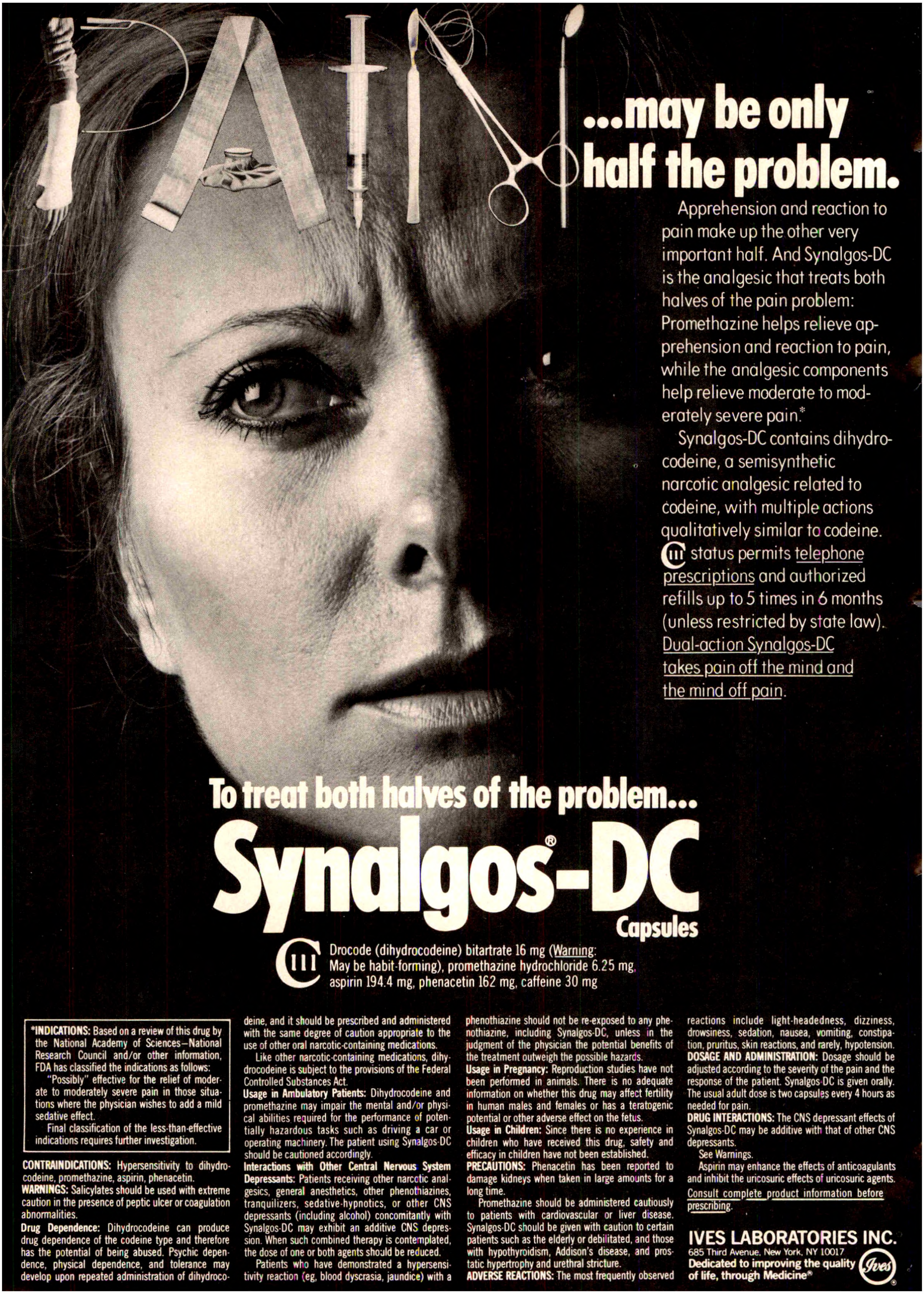

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American Journal of Obstetrics and Gynecology

volume 138 *number* 7 *part* 1 DECEMBER 1, 1980

OBSTETRICS

Measure of contraction strength in labor: Area and amplitude

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A retrospective, off-line study of the relationship of the area under the intrauterine pressure waveform (Area) to amplitude was performed on contractions from patients in normal labor and hypocontractile labor before and during oxytocin therapy. A linear correlation of Area and amplitude can be identified in the study of large numbers of contractions. Unfortunately, the wide scatter of the values of either measure for a narrow range of values of the other precludes the use of Area as a clinically useful aid in assessing hypocontractility or oxytocin response from small numbers of contractions. (AM. J. OBSTET. GYNECOL. 138:727, 1980.)

THE EVALUATION of uterine contractility is one of several important diagnostic measures used to select treatment in patients in whom labor fails to achieve adequate rates of dilatation or descent. The area under the curve of the intrauterine pressure waveform (Area) has been suggested as an acceptable substitute for the more traditional parameters, amplitude and repetition frequency, and calculation of Montevideo units.[1-4] These proposals have surfaced because of the relative technical simplicity required to obtain measure of the Area.[5, 6] In contrast, objective on-line measurement of amplitude, repetition frequency, and calculation of Montevideo units has not been achieved by a clinically useful method.

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Received for publication May 1, 1980.

Accepted August 13, 1980.

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The purpose of this study was to compare the Area with amplitude as measures of uterine contractility in normal labor and hypocontractile labor before and during oxytocin treatment. Our interest was stimulated by observations that contractions of equal amplitude had dissimilar waveform characteristics in different clinical sets.[7] Furthermore, the duration of contraction, an important determinant of Area, had a curvilinear relationship to amplitude, approaching an asymptote with contractions exceeding 40 mm Hg.[8] If our findings were valid, contractions of equal amplitudes in diverse clinical sets may have different Areas. Such possibilities would alter the clinical utility of Area, and suggest that a thorough examination of this measure should precede the incorporation of it into commercially available labor monitors.

Material and methods

The methods used are identical to those reported,[8] with the following changes: (1) the threshold for the rate of pressure change used to identify the start and end of a contraction was reduced to ±0.25 mm Hg · s^{-1}; (2) the intrauterine pressure data were

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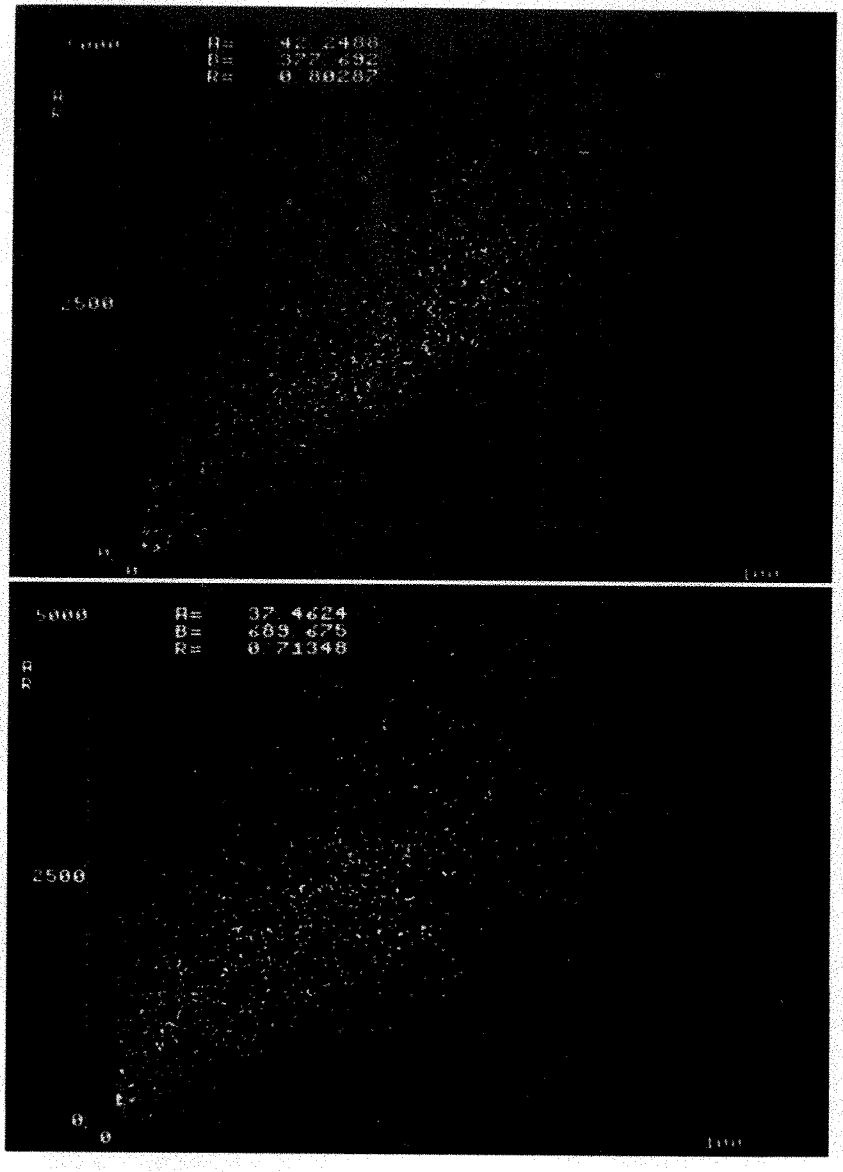

Fig. 1. Oscillographic display of Area *(AR)* in mm Hg · s units (scale = 0-5,000) and amplitude in mm Hg (scale = 0-100). *R* = linear coefficient of correlation; *A* = slope; *B* = Y intercept. *Top:* Normal labor. *Bottom:* Hypocontractile labor.

filtered in an attempt to remove unwanted pressures produced by striated muscle activity [3 db at 0.07 Hz][9]; (3) the area for each contraction was calculated by measuring the intrauterine pressure at each succeeding second, averaging each pair of successive pressure values, subtracting the initial pressure from each average, and summing the remainders; and (4) the period for each contraction is the number of seconds between the maximum amplitude of that contraction and the maximum amplitude of the antecedent contraction.

The 21 normal patients were in the acceleration or active phase of labor with normal rates of dilatation; they had received no medication other than drug analgesia, and provided 819 contractions for study. The 12 patients with hypocontractile labor manifested arrest of dilatation in the active phase of labor. The maximum dose of oxytocin used was 4 mU per minute or less in nine patients, 5.5 mU/min in two, and 15 mU/min in one. The arrest of dilatation in the patients with hypocontractility was not associated with regional anesthesia or drugs, such as $MgSO_4$, and an adequate dose of oxytocin resulted in normal rates of dilatation and vaginal delivery. The 724 contractions included in this group were obtained from the initiation of monitoring to the end of the first stage of labor and included 215 contractions prior to and 509 contractions during the administration of oxytocin.

Results

The data for amplitude, period, Area, and Montevideo units (Table I) indicate that the inclusion of many contractions associated with failure of labor to progress results in reduced mean values for amplitude and elevated values for the period. Photographs of the oscillograph display of the X/Y plots of Area and amplitude demonstrate the wide range of values for either parameter for any selected value of the other (Fig. 1).

Segregation of the data by 10 mm Hg class intervals of the amplitude (Table II) demonstrates that, for contractions ≧15 mm Hg, there are no differences in the mean Area: amplitude relationship between normal and hypocontractile labor, and Area increases with increasing amplitude. The class interval of contractions 5 to <15 mm Hg has greater Area and duration in hypocontractile labor. The prolongation of weak contractions in hypocontractile labor has been described previously[10] and results in the larger Area. In the class interval of contractions ≧65 mm Hg, the contractions are shorter, the results of shortened start-up time when the uterus is overstimulated with oxytocin.[7]

Comment

These data demonstrate that when large numbers of contractions are examined, amplitude and the area under the pressure waveform appear to have a linear relationship as suggested by the coefficients of correlation (Fig. 1). However, study of the data for small ranges of the amplitude (Table II) reveals large deviations of the Area values. The frequency distributions of the Area for three noncontiguous class intervals of the amplitude illustrate this wide scatter (Fig. 2). The inability of Area values to predict amplitude can be assessed in practical terms by estimating the probabilities of selecting class-interval values of the amplitude from a specific range of Area values. For example, with use of the data for the number of contractions in each class interval from Table II and the frequency distribution values in Fig. 2, the odds that a contraction with an Area value of 2,000 to 2,400 mm Hg · s will fall into the amplitude class intervals of 55 to <65, 35 to<45, or 15 to <25 mm Hg are 2.9 : 4.4 : 1.0, respectively. For an Area value between 2,400 and 2,800 mm Hg · s, the odds are 15 : 6 : 1.

The average Area does increase as the average amplitude increases. The sum of the Areas of large numbers of consecutive contractions does provide an estimate of the force generated by the uterus, as will the sum of the amplitudes.[11] Unfortunately, the variability of the Area values for any small range of amplitudes precludes the use of Area for identifying uterine hypocontractility or for prompt recognition of appropriate responses to oxytocin from small numbers of consecutive contractions. The latter is illustrated by Beazley,[12] who administered oxytocin for the purpose of inducing labor by doubling the dose each half-hour until the Area values identified the plateau of uterine response. The maximum dose administered was two to four times that necessary, thus illustrating the inefficient identification of the quality of uterine contractility by Area measurement.

If the purpose of quantitation of contraction strength is to recognize hypocontractility and to restrict

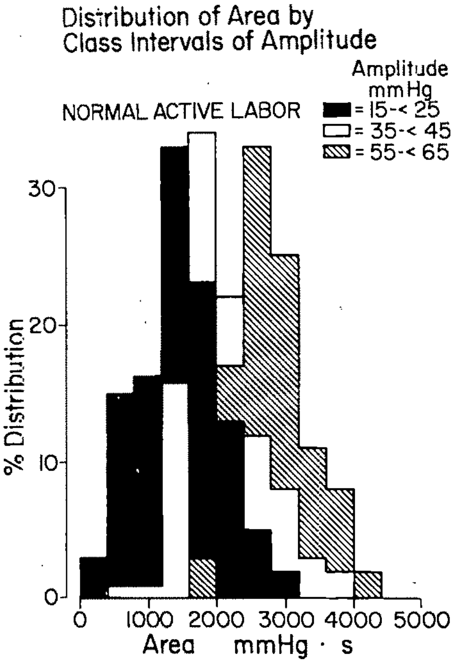

Fig. 2. Area frequency distributions (by %) grouped in binwidths of 400 mm Hg · s for each of three class intervals of the amplitude, 15 to <15, 35 to <45, and 55 to <65 mm Hg. Note that the mode group of Area values for each class interval of the amplitude never exceeds 33% or 34% of the total population, and the marked overlapping of the Area values for these noncontiguous class intervals of the amplitude.

Table I

	Normal labor (average ± SD)	*Hypocontractile labor (average ± SD)*
Amplitude (mm Hg)	42 ± 19	35 ± 20
Period (s)	144 ± 76	163 ± 95
Area (mm Hg · s)	2,154 ± 1,001	2,007 ± 1,050
Montevideo units	203 ± 137	167 ± 146
Duration (s)	69 ± 22	66 ± 23

Table II

Class-interval amplitude (mm Hg)	*Normal labor*			*Hypocontractile labor*			*P*	
	No. of contractions	*Area, mm Hg · s (average ± SD)*	*Duration, s (average)*	*No. of contractions*	*Area, mm Hg · s (average ± SD)*	*Duration, s (average)*	*Area*	*Duration*
5-<15	102	596 ± 464	29.7	144	907 ± 538	39.3	0.001	0.001
15-<25	61	1,374 ± 515	56.0	117	1,527 ± 586	62.0	NS	NS
25-<35	113	1,770 ± 602	69.0	115	1,885 ± 745	70.2	NS	NS
35-<45	154	2,120 ± 641	73.5	119	2,303 ± 698	76.8	NS	NS
45-<55	170	2,535 ± 677	78.3	110	2,495 ± 814	76.2	NS	NS
55-<65	135	2,800 ± 579	81.3	59	2,942 ± 940	77.8	NS	NS
≧65	84	3,396 ± 704	83.6	60	3,415 ± 1,018	77.1	NS	0.01

the dose of oxytocin to that necessary minimum which will achieve uterine forces found in progressive first-stage labor from the analysis of several successive contractions, it is obvious that Area is less useful than amplitude, and should not be used to repace amplitude despite the ease of quantitation. What is needed are investigations to determine whether on-line quantitation of uterine contractility, with the use of traditional measures, can identify the minimal dose of oxytocin necessary to restore normal contractility more efficiently than the current practice of the visual scanning of many feet of oscillograph paper and estimating the quality of the "contractile pattern."

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Automated erythrocytopheresis for sickle cell anemia during pregnancy

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Automated erythrocytopheresis allows for selective removal of erythrocytes from whole blood by means of extracorporeal centrifugation and separation. Remaining cellular and liquid fractions are returned to the patient, along with donor erythrocytes. Supertransfusion with the use of plasmapheresis and simultaneous direct transfusion of erythrocytes increases the hematocrit while maintaining stable intravascular volume. This technique was used in the prophylactic exchange transfusion in eight pregnant women with sickle hemoglobinopathies. Two patients required repeat procedures 7 and 13 weeks later for hemoglobin A concentration or hematocrit less than 25%. The results from the first five pregnancies are detailed. All pregnancies were carried to term, with the delivery of healthy infants. There was no fetal or neonatal morbidity, and the only maternal morbidity was a case of puerperal endometritis. Erythrocytopheresis has been established as a flexible and useful means of managing gravid patients with sickle hemoglobinopathies. (AM. J. OBSTET. GYNECOL. 138:731, 1980.)

THE PROPHYLACTIC use of partial exchange transfusion in pregnant patients with sickle hemoglobinopathies appears to improve both maternal and fetal outcome.[1] Blood rich in hemoglobin (Hb) S is removed and replaced with Hb A containing erythrocytes. The procedure can be used not only to replace cells which contain Hb S and are likely to undergo sickling, but also to elevate hematocrit and Hb A and to suppress the production of bone marrow of cells containing Hb S.

Published protocols[1, 2] outline a manual exchange of whole blood phlebotomized from the patient with packed, washed erythrocytes. The technique has proved to be effective but is time consuming, tedious, and technically cumbersome and requires a minimum hospitalization of 48 hours. The procedure calls for intravascular expansion with isotonic alkalinizing solutions prior to phlebotomy. The blood withdrawn and discarded includes both cellular and liquid fractions. Discarded along with the Hb S–containing erythrocytes are leukocytes, thrombocytes, plasma, and various humoral factors responsible for coagulation and immunologic competence.

Automated erythrocytopheresis is a technique by means of which erythrocytes can be selectively removed from whole blood with the use of extracorporeal, differential, centrifugal cell separation. Plasma and remaining cellular blood components are returned to the patient's circulation, along with an infusion of packed, washed, buffycoat-poor donor erythrocytes, while balanced withdrawal and return rates are maintained. Thus, blood volumes can be electronically controlled so that the patient is protected from hypervolemia or hypovolemia. Erythrocytopheresis can be performed as an outpatient procedure. This preliminary report details our experience with 10 procedures in eight pregnant women and demonstrates the efficacy of the exchange in increasing Hb A concentration and hematocrit.

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Received for publication April 11, 1980.

Revised July 28, 1980.

Accepted August 1, 1980.

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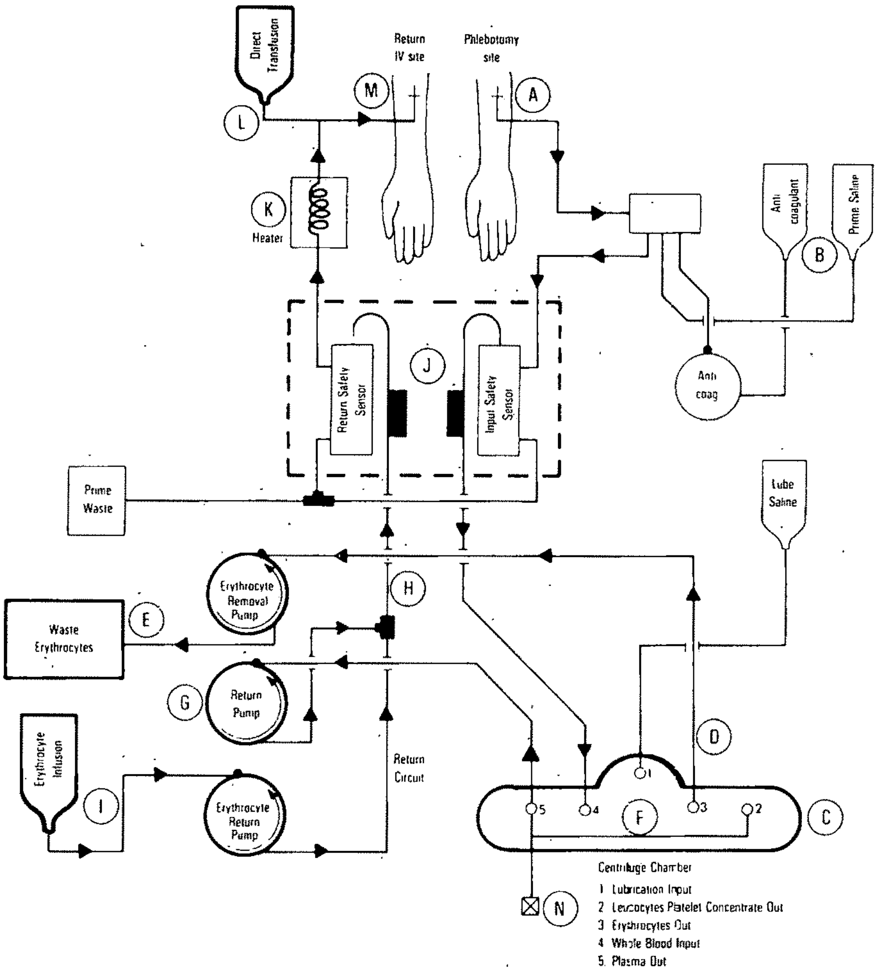

Fig. 1. Schematic diagram of erythrocytopheresis with use of an IBM 2997 Blood Cell Separator. See text for explanation.

Material and methods

Between March 21, 1979, and November 1, 1979, eight pregnant patients with clinically significant sickle hemoglobinopathies were managed at the Medical University of South Carolina. All patients were managed prospectively and by protocol. Detailed counseling was provided and informed consent was obtained.

All patients were hospitalized on the day of the procedure. A battery of laboratory studies was performed before and after each procedure (Table I). Total blood and erythrocyte volumes were calculated on the basis of surface area.

Erythrocytopheresis was performed with the use of an IBM 2997 Blood Cell Separator* altered for this specific purpose.

The procedure was performed as indicated in Fig. 1. Whole blood was removed from the patient by phlebotomy at a controlled rate of 15 to 60 ml/min *(A)* and anticoagulated with ACD-A* solution *(B)*. The whole blood was centrifuged at 600 to 1,000 rpm in a special chamber *(C)* which allowed for constant port sampling. The separated erythrocyte pool was removed and discarded after weighing *(D* and *E)*. The plasma, leukocytes, and thrombocytes were combined *(F)* and placed in a return circuit *(H)* by way of a roller pump *(G)*. An infusion of washed, buffy coat–poor, HB A–containing erythrocytes was added to the return circuit by way of another roller pump *(I)*. Phlebotomy flow and return infusion rates were electronically balanced by paired solenoid valves placed on each line *(J)*. The returned blood was warmed to body temperature

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*IBM Corporation, Biochemical Operations, Princeton, New Jersey.

*Anticoagulant citrate-dextrose solution—Formula A, Fenwal Laboratories, Division of Travenol Laboratories, Deerfield, Illinois.

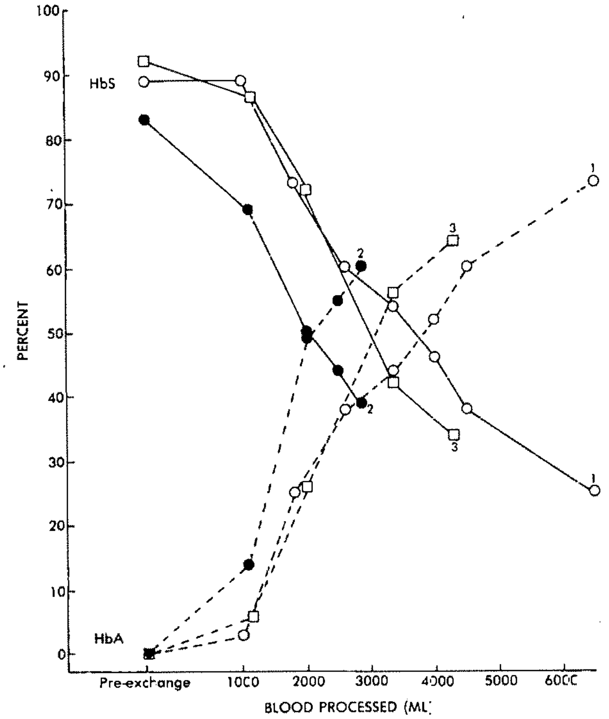

Fig. 2. Changes in concentrations of hemoglobins A and S related to blood volume processed in first three patients who underwent erythrocytopheresis.

(*K*) prior to infusion (*M*). Direct transfusions were added terminally (*L*). Plasma could be removed selectively and discarded by diverting the plasma fraction away from return pumps (*N*).

Each patient was evaluated prior to the procedure, and the blood volumes to be processed were predetermined on the basis of hematocrits, blood volumes, and Hb S concentrations. Supertransfusion that utilized direct terminal erythrocyte transfusion and simultaneous plasma withdrawal was used to elevate the hematocrit while maintaining static total intravascular volume.

Samples of blood were obtained serially throughout the procedure and analyzed for hemoglobin concentration, hematocrit, and hemoglobin electrophoretic pattern.

Soon after prenatal care was begun, the initial erythrocytopheresis was performed. All patients were followed up in a high-risk, hematology subspecialty clinic with serial hemoglobin concentrations, hematocrits, and hemoglobin electrophoreses. The procedure was repeated if the hematocrit or the Hb A concentration fell below 25%. High-risk obstetric monitoring with fetal stress and nonstress testing and determination of plasma estriol was performed at the discretion of the responsible physician. All pregnancies were allowed to progress to term unless such a course was contraindicated by fetal or maternal factors. The mode of delivery was determined from obstetric indications.

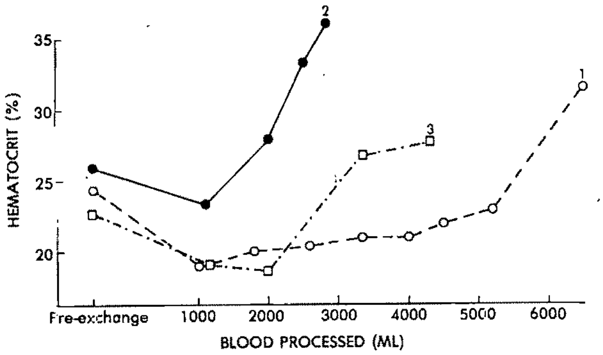

Fig. 3. Changes in hematocrits related to blood volume processed in first three patients who underwent erythrocytopheresis.

Table I. Laboratory studies performed before and after each erythrocytopheresis

Complete blood count
Platelet count
Hemoglobin electrophoresis
Serum haptoglobin
Electrolytes
Sequential multiple analyzer 12/60
Creatinine
Serology for syphilis
Serology for hepatitis B antigen
Prothrombin time
Partial thromboplastin time
Fibrinogen
Indirect and direct Coombs tests
Urinalysis

Results

Ten erythrocytopphereses were performed on eight patients (Table II). Seven patients were homozygous for Hb S, and one patient was heterozygous for both Hb S and Hb C. The gestational age at the time of the first erythrocytopheresis varied from 17 to 30 weeks. Two patients who underwent initial erytrocytophereses at 17 and 18 weeks' gestation required exchanges 7 and 13 weeks later. In the other six patients, a single erythrocytopheresis was used during the gestation.

Table II outlines the changes effected in each procedure. In seven cases, the hematocrit was significantly ($p < 0.01$) increased by the procedure. The initial hematocrit of 33.1% in Case No. 8 changed only minimally. The other two patients (Cases Nos. 5 and 6) received only a single unit of blood by terminal direct transfusion. All patients experienced significant increases in Hb A concentrations. After the automated exchange, all patients had Hb A concentrations above

Table II. Hematologic changes resulting from erythrocytopheresis

Case No.	Gestational age (wk)	*Hematocrit*		*Hemoglobin electropheresis pattern*									
				Before procedure					*After procedure*				
		Before procedure	*After procedure*	A_1	A_2	*S*	*F*	*C*	A_1	A_2	*S*	*F*	*C*
1.	28	24.4	31.0	0	2	92	6	0	73	2	25	0	0
2.	28	26.0	36.0	0	1	83	16	0	60	1	39	0	0
3.	28	23.0	27.8	0	2	93	5	0	64	2	34	0	0
4.*	17	21.2	28.0	0	2	84	14	0	65	1	34	0	0
5.†	18	26.7	27.7	0	2	70	28	0	69	2	29	0	0
6.	30	26.6	24.5	0	2	89	9	0	51	2	47	0	0
7.	24	18.5	28.0	0	2	92	6	0	68	2	30	0	0
8.	29	33.1	33.8	0	0	47	3	50	56	9	22	9	22
9.	31	26.2	32.1	21	2	68	9	0	70	2	23	5	0
10.	24	23.6	35.8	22	2	76	0	0	70	2	28	0	0

*Case No. 4 had a repeat exchange 7 weeks later, which is represented as Case No. 7.

†Case No. 5 had a repeat exchange 13 weeks later, which is represented as Case No. 9.

Table III. Fluid volume and erythrocyte changes resulting from erythrocytopheresis

Case No.	*Calculated blood volume*	*Calculated erythrocyte volume*	*Erythrocyte volume removed*	*Erythrocyte volume infused*	*Plasma volume removed*	*Erythrocyte volume directly transfused*	*Crystalloid volume infused*	*Net erythrocyte change*	*Net volume change*
1.	3,750	1,087	1,520	1,553	780	473	2,150	+506	+1,876
2.	3,825	995	870	910	800	625	475	+665	+340
3.	4,150	955	1,100	1,067	700	690	620	+657	+577
4.*	3,878	814	875	875	537	537	435	+537	+435
5.†	3,825	1,021	480	1,040	332	262	435	+822	+435
6.	5,300	1,378	1,418	1,418	330	330	570	+330	+570
7.	3,950	730	940	946	1,525	1,363	750	+1,369	+594
8.	3,875	1,314	1,578	1,577	1,183	605	595	+604	+16
9.	3,925	1,028	1.500	1,500	1,000	625	615	+625	+240
10.	3,725	857	1.187	1,187	1,065	596	465	+596	−4

*Case No. 4 had a repeat exchange 7 weeks later, which is represented as Case No. 7.

†Case No. 5 had a repeat exchange 13 weeks later, which is represented as Case No. 9.

50%, and all but two had Hb A concentrations above 60%. Each procedure required 6 to 8 U of erythrocytes.

Hematocrits from the waste erythrocyte pool (*E* in Fig. 1) ranged from 70% to 80%. Conversely, the calculated hematocrits of the "blood" returned to the patient ranged from 52% to 55%.

The dynamics of the procedure are shown in Table III. The erythrocyte volume removed correlated with the change effected in the hemoglobin electrophoretic pattern. The volume of terminal erythrocyte transfusions and the volume of plasma removed (supertransfusion) correlated with the change in hematocrit.

All patients, except Case No. 5, had erythrocyte volumes removed and replaced by almost equal volumes of Hb A–containing erythrocytes. In Case No. 5, the erythrocyte volume replaced was 210% of that removed, and a single unit of erythrocytes was transfused terminally. Excellent Hb A concentration was achieved (69%), but there was no significant change in hematocrit.

Case No. 1 had a positive change in net volume of 1,876 ml with some 2,150 ml infused as crystalloid. Later analysis identified this potentially dangerous problem, and steps were taken to restrict this volume to the necessary minimums required for priming, anticoagulation, lubrication, and maintenance of intravenous lines. Subsequently, an average change in net volume of 503 ml occurred.

Changes in net volume are dependent on multiple variables, including the volumes removed and infused throughout the procedure and the extent of supertransfusion (direct transfusion plus removal of plasma). Also, infusions of crystalloid may effect significant changes in net volumes, as in Case No. 1. The disparity between the effective hematocrit of the blood removed and that of the blood replaced favors a net positive change in intravascular volume.

There were no adverse reactions during the proce-

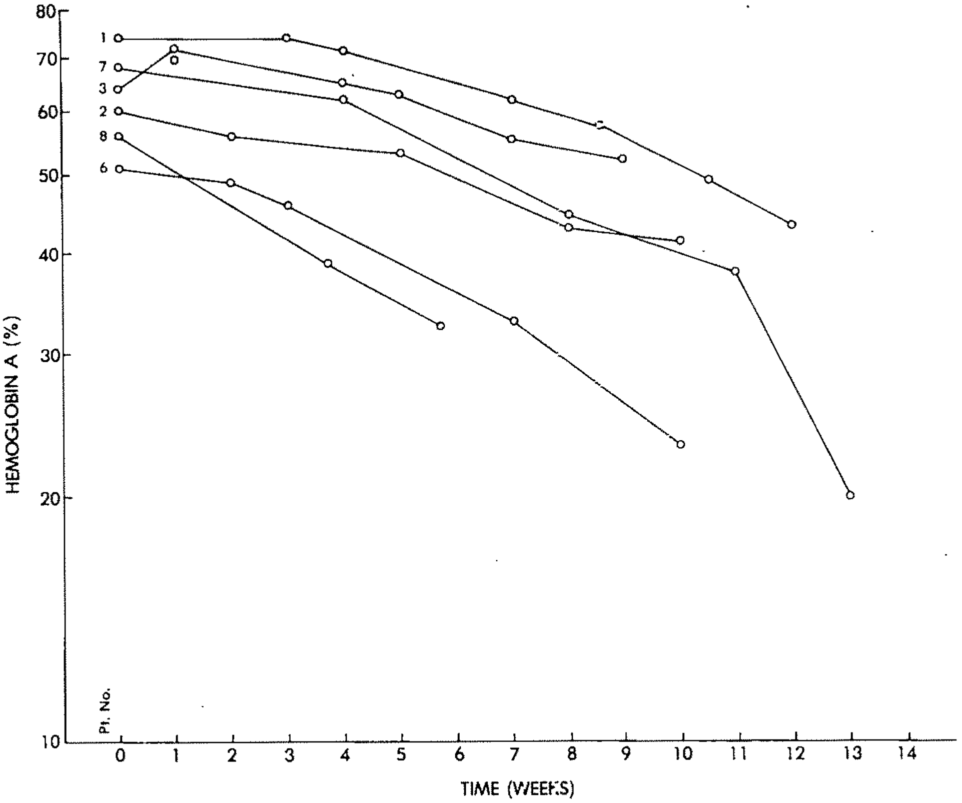

Fig. 4. Serial changes in hemoglobin A concentration in six patients who underwent erythrocytopheresis.

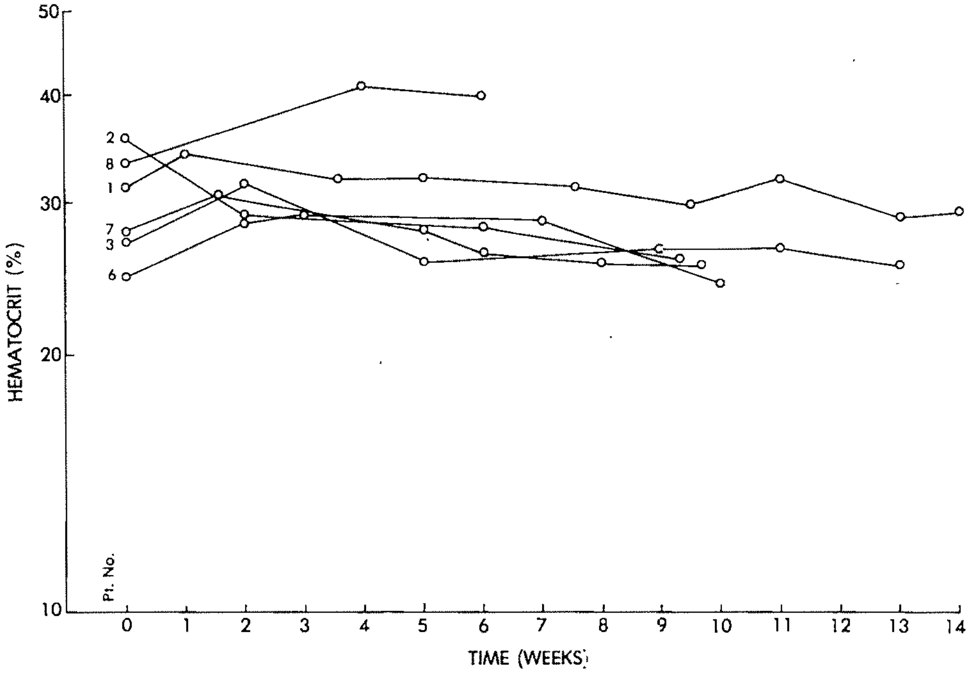

Fig. 5. Serial changes in hematocrit in six patients who underwent erythrocytopheresis.

dure. All patients remained comfortable and had stable vital signs. One patient (Case No. 1) diuresed briskly after the fluid overload previously discussed.

No significant change or trend was demonstrated on evaluation of coagulation profiles, electrolytes, and calcium and serum protein concentrations. All patients entered the protocol with negative indirect Coombs tests. One patient (Case No. 4) later demonstrated multiple warm and cold antibodies that were thought to have been present prior to the original erythrocytopheresis but undemonstrated. She hemolyzed the administered cells over a period of 14 days and underwent a second erythrocytopheresis 7 weeks later.

Fig. 2 illustrates the changes in concentrations of Hb A and Hb S which occurred during the first three procedures. There appeared to be an initial latent phase during the processing of the first 1,000 ml of blood. The concentrations of Hb A and Hb S remained static during that phase. Fig. 3 reveals that, in these same cases, a significant drop in hematocrit was noticed simultaneously. This phenomenon was thought to be secondary to the loss of 250 to 300 ml of erythrocytes required to fill the reservoir of the IBM Blood Cell Separator. The patient's intravascular volume remained stable but the erythrocyte volume fell, as shown in Fig. 3. After the latent phase, the efficacy of the procedure resulted in rapid exchanges of Hb S cells with cells rich in Hb A. The addition of terminal transfusion and plasma removal (supertransfusion) resulted in terminal elevation of hematocrit.

Six patients were followed long enough to demonstrate the decay of Hb A concentration and hematocrit (Figs. 4 and 5). Hematocrits remained relatively stable, whereas Hb A concentrations gradually fell from the time of the procedure, as anticipated from the known half-life of transfused blood. At approximately 7 to 9 weeks, the rate of decay of Hb A concentration appeared to increase, possibly reflecting the re-emergent production by bone marrow of cells containing Hb S. As previously described, only two patients required repeat procedures because of a significant fall in either Hb A concentration or hematocrit.

Initial erythrocytophereses required 3 hours and constant physician attendance, but with increased experience, physician time was minimized. More recent exchanges have required approximately 1.5 hours.

Delivery has occurred in five of the eight pregnancies. All infants were born at term, and no pregnancies were interrupted for maternal or fetal indications. The weight of the infants ranged from 3,015 to 3,171 grams, with a mean of 3,111 grams. All infants had Apgar scores of 7 to 10 at 5 minutes, enjoyed normal hospital courses, and were discharged with their mothers.

The duration of hospital confinement for the five completed pregnancies, including erythrocytopheresis and delivery, varied from 5 to 10 days. If the normal anticipated stays for vaginal and cesarean deliveries are excluded, the confinements related to the hemoglobinopathy ranged from 2 to 4 days, with a mean of 3.4 days.

Two patients experienced mild peripartum complications. A single pregnancy was associated with light meconium staining during spontaneous labor at 41 weeks' gestation, and a single patient had mild puerperal endometritis subsequent to vaginal delivery.

Comment

Traditionally, sickle cell anemia and its severe variants are associated with high perinatal mortality rates and increased risk of maternal morbidity and mortality.[3-6] Pregnancy increases the risks of crisis, toxemia, and infection and may lead to long hospital confinements. Fetal welfare is jeopardized by high abortion rates, growth retardation, and prematurity. Pregnancy places physiologic demands on a pathologically compensated individual, resulting in deterioration of most organ systems.

Automated erythrocytopheresis has advantages over manual exchange transfusions in that blood volumes may be controlled, hematocrits selectively increased, and Hb A concentrations greater than 60% achieved through an outpatient procedure. Each patient's specific hematologic needs may be evaluated, and the procedure tailored to deliver the desired results.

Refinements in the procedure have been made, the use of maintenance fluids minimized, and the use of supertransfusion maximized to maintain static intravascular volumes and to improve hematocrits. Also, the utilization of time has been streamlined so that 1.5 hours are now required to set up the blood cell separator, perform the erythrocytopheresis, and supertransfuse.

Clinically, the initial pregnancy outcomes support the efficacy of the procedure in improving the pathologic state in sickling disorders and in leading to improved perinatal and maternal outcomes. Although the procedure has been used in only eight patients, it may lead to reduced hospital confinement, fewer simple transfusions, and elimination of other supportive therapies used in managing sickle cell anemia in pregnancy. The preliminary results are encouraging, but further clinical and laboratory evaluations are necessary before the place of erythrocytophoresis in the management of pregnant patients with sickling hemoglobinopathies can be established.

We gratefully acknowledge the assistance of Kathleen Reynolds and Betsy Gwynette in the immunohemotherapeutics laboratory in setting up the protocol and performing the procedures.

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Prolactin and the renin-aldosterone axis in human pregnancy

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In view of observations suggesting an osmoregulatory role for prolactin (PRL) and the central role of the renin-aldosterone system in the regulation of pressure-volume homeostasis, this study was undertaken to determine whether acute drug-induced alterations in circulating PRL had any effect on mean arterial blood pressure (MAP), serum osmolality, and the renin-aldosterone response during early pregnancy. Bromocriptine suppressed mean baseline PRL by 83% ($p > 0.05$), whereas chlorpromazine increased it more than twofold ($p < 0.05$) during a 180-minute study period. MAP, mean serum osmolality, plasma renin activity, and aldosterone concentrations were not significantly different from mean baseline values after the administration of either drug. These data suggest that, during early pregnancy, the renin-aldosterone axis is independent of PRL and resistant to the beta-adrenergic agonist effect of chlorpromazine. (AM. J. OBSTET. GYNECOL. 138:738, 1980.)

PROLACTIN (PRL) may be involved in the control of fluid exchange across the amniotic membrane.[1, 2] Estradiol and testosterone affect specific PRL binding in the kidneys and adrenal glands, thus suggesting that gonadal steroids participate in the PRL osmoregulatory system in the rat.[3] PRL receptors are present in the ependyma of the rat choroid plexus, thus suggesting involvement of PRL in the composition of cerebrospinal fluid.[4] Disordered secretion of PRL has also been described in disturbances of fluid and electrolyte metabolism associated with ovulation,[5, 6] hypertensive disorders of pregnancy,[7] and congestive heart failure.[8] In view of these observations and the central role of the renin-aldosterone system in the regulation of pressure-volume homeostasis, this study was undertaken to determine whether acute drug-induced alterations in circulating PRL had any effects on arterial blood pressure, serum osmolality, and the renin-aldosterone response during pregnancy.

Material and methods

The five women receiving bromocriptine (5 mg orally) had a mean age of 21.4 years (range, 19 to 23 years) and mean gestational age of 13 weeks (range, 9.5 to 18.5 weeks). The five women receiving chlorpromazine (50 mg intramuscularly) had a mean age of 20.4 years (range, 19 to 23 years) and mean gestational age of 12.7 weeks (range, 9.5 to 17.7 weeks). All subjects who were later to have therapeutic abortions gave informed consent, were free of dietary restrictions, were not receiving medications, and were free of physical signs of disease. All studies were conducted in hospital, commencing between 0700 and 0900 hours, with the subjects in the supine position and having received nothing by mouth from midnight until completion of the study. The drugs were administered by the routes stated earlier, immediately after the second zero time sample was obtained through an indwelling cannula placed in an antecubital vein. Zero time data were a mean of two samples obtained 15 minutes apart. Plasma renin activity (PRA) was assayed by the technique of Haber and associates[9] (Dr. G. E. Wilkins, St. Paul's Hospital, Vancouver, British Columbia, Canada), and aldosterone was measured by radioimmunoassay (London Biochemical Reference Laboratory, London, Ontario, Canada). PRL was measured by radioimmunoassay, and the PRL data were previously reported.[10] Mean arterial blood pressure (MAP) = $\frac{\text{systolic BP} + (\text{diastolic BP} \times 2)}{3}$ mm Hg. Serum osmolality was measured by the Division of Clinical

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This study was supported by a grant awarded to Dr. Ho Yuen by The British Columbia Heart Foundation.

Received for publication April 24, 1980.

Revised July 15, 1980.

Accepted August 4, 1980.

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Chemistry, Department of Pathology, Vancouver General Hospital. Data were analyzed by one-way analysis of variance for overall statistical significance, and the mean from each sampling interval was compared to that of the baseline, with the use of Dunnett's test as described by Winer.[11]

Results

The results are shown in Fig. 1. After bromocriptine, mean baseline PRL concentrations declined from 60 ± 24 (SEM) ng/ml to 10 ± 3 ng/ml, 17% of baseline. This difference was not significant ($p > 0.05$). After chlorpromazine, mean PRL was 53 ± 10 ng/ml, increasing significantly ($p < 0.05$) between 60 and 120 minutes, with peak values of 124 ± 16 ng/ml at 90 minutes. No significant differences in mean arterial blood pressure, PRA, aldosterone, and serum osmolality were observed after the administration of either bromocriptine or chlorpromazine.

Comment

During early human pregnancy, marked drug-induced alterations in maternal PRL concentrations failed to alter MAP, serum osmolality, or the renin-aldosterone system over a 3-hour study interval. In contrast to the nonpregnant state,[12] chlorpromazine failed to significantly affect the renin-aldosterone axis during early pregnancy. The mean level of PRA (but not of aldosterone) was higher after chlorpromazine; at 180 minutes, it was 65% above baseline, but this was statistically not significant. The ability of chlorpromazine to stimulate increases in circulating PRA and aldosterone in nonpregnant subjects has been attributed to the beta-adrenergic agonist effect of this phenothiazine,[12, 14, 15] although other possibilities, including stimulation of PRA by a centrally mediated drug effect on the autonomic nervous system or directly via the adrenal medulla, have not been excluded.[12] In our experiments, twofold to fivefold higher doses of the drug were given intramuscularly, as compared to the doses administered intravenously by Robertson and Michelakis[12] in nonpregnant subjects. The effect of differing routes of administration of chlorpromazine on the results obtained in these studies is not known, and may be of little significance because the clinical effect by either route is immediate, with high levels detected in the blood at 1 hour and maintained for 12 hours after an intramuscular injection of doses that we employed in these experiments.[15]

The secretion of aldosterone from the adrenal glomerulosa appears to be under maximal tonic dopaminergic inhibition, via mechanisms independent of the renin-angiotensin system.[13] Bromocriptine is a specific dopaminergic agonist which lowered mean maternal plasma PRL levels to 17% of baseline. However, because of wide variation and a limited number of samples, this suppression was statistically not significant. Nevertheless, the drug achieved a reduction in mean PRL levels of 83%, without significantly affecting

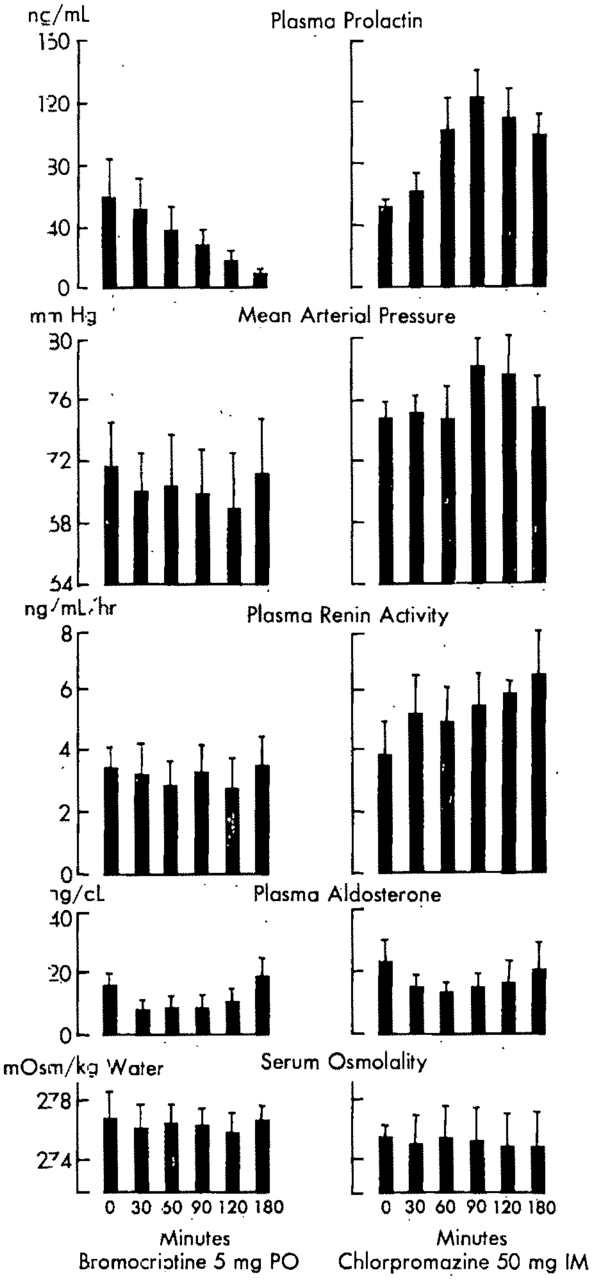

Fig. 1. Plasma prolactin, mean arterial blood pressure, plasma renin activity, plasma aldosterone, and serum osmolality in women receiving bromocriptine (n = 5) and chlorpromazine (n = 5) in doses and routes of administration as noted. Except for prolactin at 60, 90, and 120 minutes after chlorpromazine ($p < 0.05$), no significant differences were observed in all other values after either drug.

MAP, mean serum osmolality, PRA, and aldosterone. This is also compatible with the belief that the maximal tonic dopaminergic inhibition of aldosterone secretion, which is thought to occur in the nonpregnant state,[13] persists during early pregnancy.

Our results, taken together with the findings reviewed, suggest that, under the conditions of our experiments performed in early pregnancy, the renin-aldosterone axis is both independent of PRL and resistant to beta-adrenoreceptor stimulation. Augmented urinary excretion of epinephrine and norepinephrine,[16] unaccompanied by increased circulating levels of PRA, angiotensin, and aldosterone[17] in pregnancy-induced hypertension, further suggests that this apparent insensitivity of the renin-aldosterone axis persists during these clinical disorders of late pregnancy. These data, however, do not exclude a long-term effect of altered PRL secretion on the parameters which we studied, especially when sex steroid concentrations in plasma are known to be augmented.

We wish to thank Miss Wendy Cannon, B.Sc., for expert technical assistance, Miss Suzanne Woolley, R.N., for assistance in data analysis and the clinical aspects of the project, and Mr. A. Nalecz, Sandoz Company, Montreal, Quebec, Canada, for supplies of bromocriptine.

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Increased pressor activity in incubated plasma of pregnant rabbits

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A study to measure the pressor activity which is generated in incubated plasma of pregnant and nonpregnant rabbits was performed by means of a recently modified bioassay technique. It was found that plasma of normal pregnant rabbits generated significantly higher amounts of active pressor principle (APP) than those generated by plasma of nonpregnant rabbits. The possible relevance of these results to blood pressure regulation during normal and abnormal pregnancies is discussed. (AM. J. OBSTET. GYNECOL. 138:741, 1980.)

INCUBATED PLASMA of several species has been shown to have a pressor activity which is thought to be caused by a nondialyzable active pressor principle (APP).[1] APP has been recently characterized as a lysophosphatidic acid bound to albumin.[2] Its biological and physicochemical properties clearly distinguish it from the presently known vasoactive substances.[1, 3, 4] The physiologic role of APP is not yet known, but increased APP levels have been found in patients with hypertensive nephritis,[5] pre-eclampsia,[3] and malignant essential hypertension.[6] These findings indicate that APP could play a role in blood pressure regulation. Gordon and Rieske,[4] using a recently modified bioassay technique, reported that plasma from rabbits contains abundant APP with physicochemical characteristics similar to those found in humans. Since the pregnant rabbit is frequently used to study hypertension of pregnancy,[7] we investigated whether normal pregnancy in these animals is characterized by increased APP.

Animal preparation

A modification of a previously described assay method[1] for APP was used in these studies. White Wistar rats of either sex, weighing 250 to 350 gm, were anesthetized by an intraperitoneal injection of 30 mg/kg of pentobarbital (Nembutal). Anesthesia was maintained by a continuous intraperitoneal infusion of pentobarbital at a constant rate of 25 mg/kg/hr. Rectal temperature was recorded and kept between 37° and 38° C. The trachea was cannulated with a 240-PE* catheter and oxygen was blown past the catheter opening at a rate of 0.6 L/min. A femoral artery and both femoral veins were exposed, and each was cannulated with a PE-50† catheter. The arterial cannula was connected to a Beckman Dynograph 550 monitor, and pulse pressure, mean arterial pressure (MAP), and heart rate were continuously recorded (Fig. 1). Pentolinium (Ansolysen, Wyeth) was administered intravenously at a constant rate of 0.2 mg/kg/hr. Injections of plasma test samples were started an hour after the completion of surgical procedures and blood pressure stabilization. Plasma samples to be assayed were intermittently injected as a bolus through the second venous cannula every 8 to 10 minutes and the catheter was flushed with 0.2 ml of saline.

Preparation of plasma for APP assay

Blood samples (5 ml) were obtained in a sterile manner from nonpregnant and pregnant (28 to 31 days' gestation) New Zealand white rabbits and immediately centrifuged in heparinized tubes at 4° C (10 minutes at 2,000 × g). The plasma was then incubated for different periods, as described below, at 37° C, in a Napco 220 water bath, and stored frozen at −20° C until assayed.

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Received for publication July 16, 1980.

Accepted August 13, 1980.

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*Inside diameter, 1.67 mm; outside diameter 2.42 mm.
†Inside diameter, 0.58 mm; outside diameter 0.965 mm.

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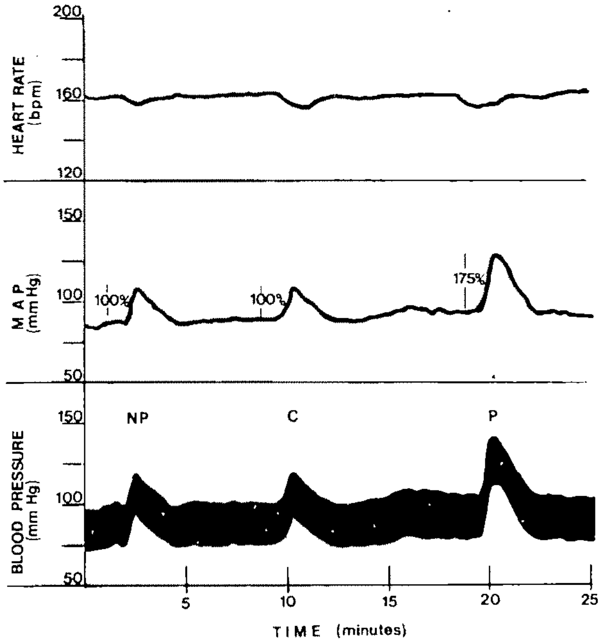

Fig. 1. Tracing of an experimental record showing changes in blood pressure, MAP, and heart rate (beats per minute), after injection of a plasma sample of nonpregnant *(NP),* control *(C),* and pregnant (P) rabbits. The relative pressor response (percent) is indicated near the MAP amplitudes.

Preliminary experiments were performed to establish the incubation time and volume of plasma which would elicit a suitable pressor response in this rat preparation. Samples from 10 nonpregnant rabbits were incubated for periods ranging from 4 to 72 hours and an incubation time–relative pressor response curve was constructed (Fig. 2). Ten additional samples were then obtained from 10 different nonpregnant animals and incubated for 24 hours. Plasma volumes ranging from 0.1 to 0.4 ml/kg were assayed and a dose-relative pressor response curve was constructed (Fig. 3). After completion of this preliminary study, it was decided that 0.2 ml/kg of plasma, incubated for 24 hours, would be used for assay purposes.

Plasma samples were then obtained from 10 pregnant and 10 nonpregnant rabbits and incubated, and each sample was tested in five rats. Relative pressor response was assessed as previously described by Wurm and Kenner.[1] Briefly, the amplitude of the rise in MAP caused by each test sample was compared to that caused by a standard control sample. This control sample was taken from a pool of 24-hour incubated rabbit plasma, obtained from one nonpregnant rabbit. The pressor response to a 0.2 ml/kg dose of the standard pool plasma was set at 100%. This pressor response corresponds to a MAP rise of approximately 25 mm Hg above the baseline value (Fig. 1). Control samples were injected several times in each experiment to assure constant sensitivity of each rat assay preparation. The results are expressed as a mean ± standard deviation of the mean, and the means were compared by the unpaired Student's t test.

Results

The increase in pressor activity of rabbit plasma was directly proportional to the duration of incubation. A peak in pressor activity was reached with plasma incubated for 48 hours and there was no further increase thereafter (Fig. 2). In the range of plasma volumes tested (0.1 to 0.4 ml/kg), a linear correlation between the volume injected and the pressor response was observed (Fig. 3).

The mean relative pressor response elicited by incubated plasma of 10 term pregnant rabbits was significantly higher (181% ± 23%) than that elicited by plasma of 10 nonpregnant animals (98.6% ± 17%) (Fig. 4).

Comment

Our results confirm previous reports that incubated nonpregnant rabbit plasma contains significant amounts of APP.[4] During normal gestation in the rabbit, the amount of APP is significantly increased over the nonpregnant levels. These findings are in accord with those reported by Hertlein and associates[8] in normal pregnant women. In preliminary experiments, we have confirmed that the plasma of pregnant women generates more APP than plasma from nonpregnant women or normal men. However, an important unsolved issue is the pressor activity of plasma during hypertensive pregnancies. Hunter and Howard,[5] who assayed human plasma in vitro, have shown an increase of pressor activity in plasma obtained from women with hypertensive pregnancies. They also noted that this pressor activity increased after incubation.[10] In a recent study, Pirani and MacGillivray[11] autotransfused puerperal women who had hypertension in pregnancy with plasma which was obtained antepartum. A significant, sustained blood pressure rise was observed in these women while no change in blood pressure occurred after autotransfusion of puerperal normotensive control subjects. The aforementioned studies may indicate that an increase in plasma pressor activity over normotensive pregnant levels occurs during hypertensive pregnancies and that this increase may be related to higher APP levels.

The source of the increased APP levels during pregnancy is unknown at present, but there is some cir-

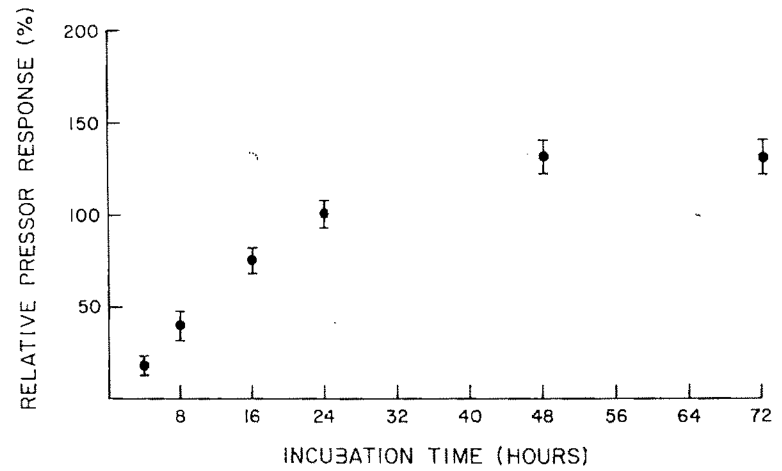

Fig. 2. The relationship between the incubation period of the rabbits' plasma samples (n = 10) and pressor activity.

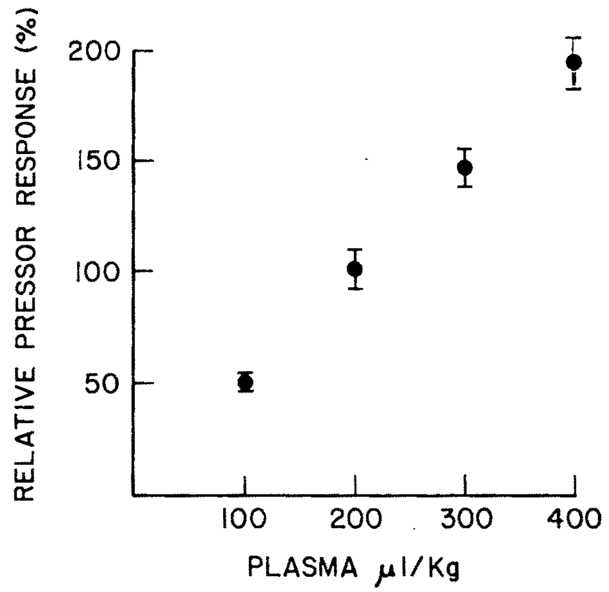

Fig. 3. The relationship between the dose of incubated rabbits' plasma samples (n = 10) and pressor activity.

cumstantial evidence that plasma draining the gravid uterus has higher pressor activity than plasma draining other organs.[9] Attempts to demonstrate that the decidua was responsible for this pressor activity were unsuccessful,[12] but the possibility of fetoplacental production has not yet been ruled out. Measurements of uterine and umbilical arteriovenous APP differences, which are currently in progress in this laboratory, may help elucidate these points.

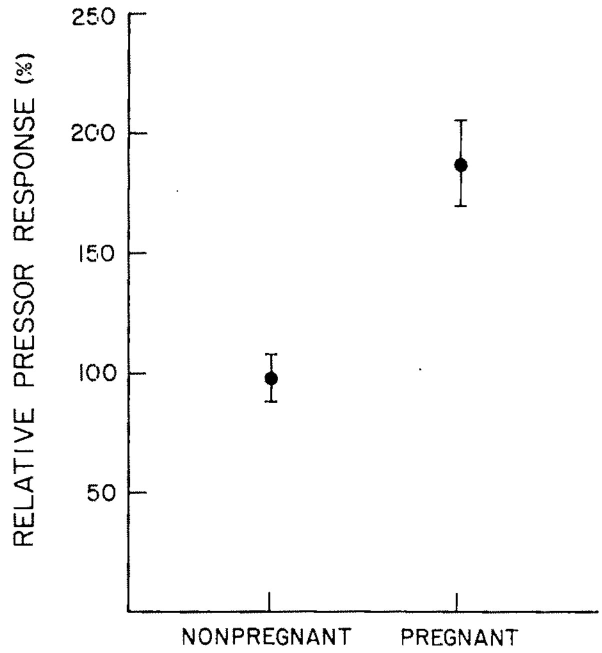

Fig. 4. Pressor response to incubated plasma samples of nonpregnant (n = 10) and term pregnant (n = 10) rabbits.

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Estimation of gestational age and maternal serum steroid concentrations: Total estriol

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Acute increases in maternal serum unconjugated estriol between 35 and 37 weeks of pregnancy have been reported to correlate predictably with gestational age. In this study total estriol was measured by radioimmunoassay in an effort to define maturational surges. Statistically significant variations from predicted regression lines were not demonstrated in any individual patient or in grouped data. (AM. J. OBSTET. GYNECOL. 138:745, 1980.)

MULTIPLE DIRECTLY and indirectly related events support an acceleration of fetal adrenal steroidogenesis in the third trimester of normal pregnancy.[1-7] Additional data have been summarized in recent reviews.[8, 9]

A statistically significant surge in the maternal serum level of unconjugated estriol at 36 weeks ± 6 days has been reported to accurately predict gestational age.[10, 11] The present study was undertaken to define the normal increase in serum total estriol from 5 to 3 weeks prior to term and to delineate any surge points.

Material and methods

Thirty-four patients were studied in a longitudinal manner following informed consent. All of the pregnancies were normal and gestational dates were accurate. Only patients whose menstrual cycles were 23 to 30 days in length and who had pelvic examinations to confirm uterine size in the first trimester were selected. Gestational age from quickening, first audible fetal heart tones, and newborn examinations were all consistent with the established estimated dates of confinement.

Thirteen of the patients were studied weekly from 32 or 33 to 38 or 39 weeks' gestation. Twenty of the remaining 21 patients were studied daily beginning at week 35 and continuing for 12 to 15 days, and the last patient was studied for 8 days. Antecubital venous blood specimens were drawn between 0900 and 1500 hours for the group. Since diurnal variation is still controversial,[12] individual patients were sampled within 1 hour of the same clock time at each visit.

Dated specimens from all patients were entered in a data book chronologically and assigned random numbers generated from a standard table. The technician performed each determination sequentially by random numbers. The chronologic numbers were rematched after each assay. All samples from an individual patient were included in a single assay with the exception that unknowns with a coefficient of variation >15% in the duplicate tubes were empirically rejected and repeated. The repeat rate was 4.6%. The median coefficient of variation between replicates for 372 unknowns was 3.8%.

Antiserum, estriol standards, and ^{125}I-labeled estriol were supplied by the Amersham Corporation, Arlington Heights, Illinois. The enzyme hydrolysis was performed according to the manufacturer's directions, but the assay was modified to include a total count tube and a nonspecific binding (NSB) tube of male serum with each run. The weighted logit-log method was used for data reduction.[13] Two quality control sera were included in each run and yielded means of 51.6 ± 7.6 and 147.4 ± 12.8 ng/ml. The within-assay coefficient of variation was 3.6% for the low control and 2.4% for the high control.[14] The corresponding figures between assays were 15.0% and 9.0%. Ten repetitions within a run of each control revealed no within-assay systematic drift.

The logarithmic value of the serum total estriol[10, 15] as calculated by the above methods was used for further data reduction in this report.

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The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Received for publication June 9, 1980.

Accepted July 30, 1980.

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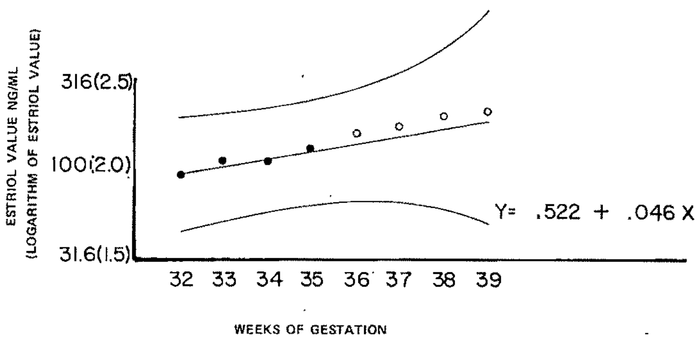

Fig. 1. Regression line (weekly data) at weeks 32 to 35 and 95% confidence interval.

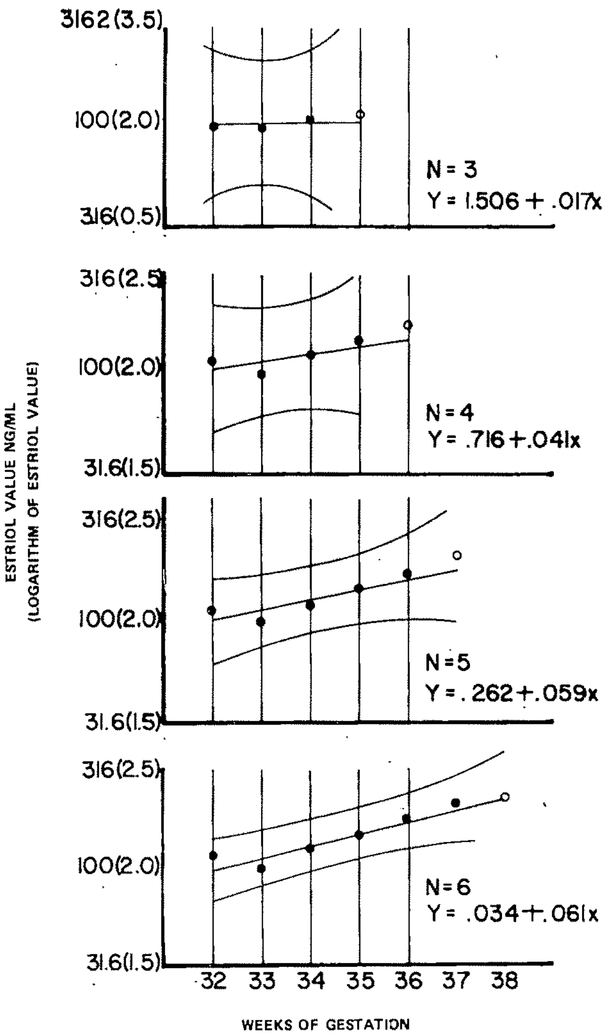

Fig. 2. Regression lines and 95% confidence intervals from 32 weeks in patient No. 9. 0 = Point not used in computation.

Results

Summary statistics from patients sampled weekly are shown in Table I and those from patients sampled daily are shown in Table II.

The calculated regression lines were Y = 0.261 + 0.053X for the weekly data and Y = 0.466 + 0.048X for the daily data. They did not differ significantly in slope or intercept.[16]

The regression line of the weekly data calculated through 35 weeks is shown in Fig. 1. The values at 36 and 37 weeks did not exceed the upper limit of the predicted 95% confidence interval for the line. Including the data for 35 weeks, 1 day and 35 weeks, 2 days in the regression and expanding the comparisons to all daily and weekly points thereafter up to 38 weeks reveals that all points excluded from the regression still fall within the 95% confidence interval of the predicted line.[17]

The apparent nadir in the data at 35 weeks, 2 days was marginally different from the apparent peak at 36 weeks, 2 days by Student's t test ($t = -1.99$, 39 df, $0.025 > P < 0.05$). However, comparisons of the slope from 32 to 35 weeks with the slope from 35 weeks to 37 weeks and the slope of combined daily and weekly data from 32 weeks to 35 weeks, 2 days with the slope from 35 weeks, 2 days to 37 weeks revealed no difference.[16]

Individual patients were studied by computing the regression line from the first three data points and iterating the search for an ensuing value which exceeded the 95% confidence interval predicted for that point in the sequence.[17] Defined in this manner, a surge occurred in none of the patients.

Comment

Although an occasional inflection point in the exponential increase was apparent between 35 and 37 weeks' gestation when individual maternal serum free estriol concentrations were studied,[18] no similar surges were noted with total estriol. With a small N of 3, minimal variability in the data from sample to sample produces very wide predicted confidence intervals, as illustrated by the patient example in Fig. 2. Note the change in scale of the Y axis needed to fit N = 3. It is apparent that although an increasing N rapidly nar-

Table I. Summary statistics from weekly samples

	Week of gestation							
	32	*33*	*34*	*35*	*36*	*37*	*38*	*39*
N	7	12	13	13	13	13	13	8
Mean (ng/ml)	95.1	117.1	121.3	136.1	161.2	179.4	206.2	213.3
S_x	26.9	36.9	44.0	38.2	45.2	45.9	54.3	47.1

Table II. Summary statistics from daily samples

	Length of gestation (weeks, days)													
	35, 1	*35, 2*	*35, 3*	*35, 4*	*35, 5*	*35, 6*	*35, 7*	*36, 1*	*36, 2*	*36, 3*	*36, 4*	*36, 5*	*36, 6*	*36, 7*
N	21	21	21	20	21	21	21	21	20	20	20	20	19	14
Mean (ng/ml)	154.8	145.3	159.7	162.7	158.4	154.4	158.3	159.8	182.2	175.6	181.5	179.0	170.1	185.6
S_x	65.6	53.9	59.2	64.2	63.0	55.4	63.0	65.5	64.5	67.3	71.0	82.5	66.9	55.6

rows the confidence interval, the week-to-week variability still produces a relatively wide prediction. More frequent sampling and pooling of multiple specimens drawn several minutes apart at each sampling[19] could yield more reproducible data. Even if these techniques could be shown to yield reliable results, this would not seem to be a practical method of estimating gestational age.

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Prostaglandin $F_{2\alpha}$ metabolites in plasma and urine during pseudopregnancy in the rabbit

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Two prostaglandin (PG) $F_{2\alpha}$ metabolites, 13,14-dihydro-15-keto $PGF_{2\alpha}$ ($dhkPGF_{2\alpha}$) in blood and $5\alpha,7\alpha$-dihydroxy-11-keto tetranorprostane 1,16-dioic acid ($PGF_{2\alpha}UM$) in urine, were measured in the pseudopregnant rabbit by radioimmunoassay. During pseudopregnancy, marked increases in the two $PGF_{2\alpha}$ metabolites were demonstrated at about the time of luteal regression. Hysterectomy and intrauterine administration of indomethacin prolonged the life span of corpora lutea in the pseudopregnant rabbit by 8 and 5 days, respectively, with no elevation of $PGF_{2\alpha}UM$ excretion. The findings indicate that $PGF_{2\alpha}$ is a uterine luteolysin in the pseudopregnant rabbit, as in other animal species. (AM. J. OBSTET. GYNECOL. 138:748, 1980.)

HYSTERECTOMY of the pseudopregnant hamster,[1] rat,[2] and rabbit[3] significantly prolongs the life span of corpora lutea. This suggests that the uterus must produce a substance, a luteolysin, which can influence the life span of corpora lutea. The luteolytic properties of exogenous prostaglandin (PG) $F_{2\alpha}$ in several animal species[4-10] and the pulsatile elevations of $PGF_{2\alpha}$ in the utero-ovarian venous blood at about the time of luteal regression in the ewe,[11, 12] cow,[13] and sow[14] indicate that $PGF_{2\alpha}$ is possibly a uterine luteolysin. No data are available comparing the increase in $PGF_{2\alpha}$ with luteolysis in the pseudopregnant rabbit.

$PGF_{2\alpha}$ metabolites have been described as being better than the parent compound as indicators for monitoring the production of $PGF_{2\alpha}$ because of rapid metabolism of PGs.[15-17] The pulsatile elevations of 13,14-dihydro-15-keto $PGF_{2\alpha}$ ($dhkPGF_{2\alpha}$) in peripheral blood[20] of the ewe and heifer, and marked increases in the excretion of $PGF_{2\alpha}$ urinary metabolite in the guinea pig[16] were reported to be observed concomitantly with luteolysis in the estrous cycle.

If $PGF_{2\alpha}$ is a uterine luteolysin in the pseudopregnant rabbit, the life span of its corpora lutea should be prolonged by indomethacin, an inhibitor of PG biosynthesis.

The present study was undertaken to determine the increase in and the site of $PGF_{2\alpha}$ production during luteal regression in the pseudopregnant rabbit by measuring $dhkPGF_{2\alpha}$ in peripheral blood and $5\alpha,7\alpha$-dihydroxy-11-keto tetranorprostane 1,16-dioic acid ($PGF_{2\alpha}UM$) in urine, and to investigate the influence of hysterectomy and indomethacin on $PGF_{2\alpha}$ production and luteal regression.

Material and methods

Animals. Eight virgin, female white Japanese rabbits 3 to 4 months old (average body weight, 3 kilograms) as a control group were injected subcutaneously with 50 μg of 17β-estradiol in 0.2 ml of sesame oil. Ovulation was induced in the estrous rabbits by a subcutaneous injection of 100 IU of human chorionic gonadotropin (hCG) in 1 ml of saline solution 3 days after administration of 17β-estradiol. The day of injection of hCG was designated as Day 0 of pseudopregnancy. Six rabbits were subjected to hysterectomy under intravenous pentobarbital anesthesia (approximately 25 mg/kg), with caution being taken not to injure the ovarian blood vessels. Ovulation was induced 3 weeks postop-

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This work was supported by The Ministry of Education, Science and Culture of Japan (Grant-in-Aid for Scientific Research 457400).

Received for publication December 6, 1979.

Accepted July 30, 1980.

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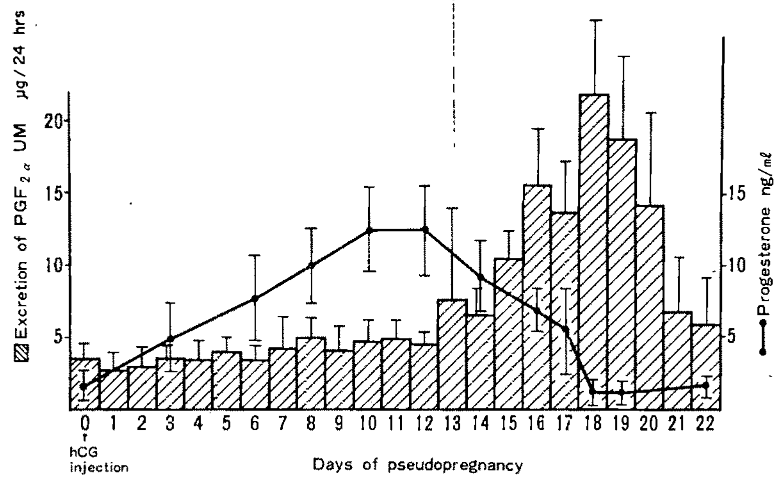

Fig. 1. Plasma progesterone level and 24-hour urinary excretion of $5\alpha,7\alpha$-dihydroxy-11-keto tetranorprostane 1,16-dioic acid ($PGF_{2\alpha}UM$) during pseudopregnancy in rabbits (n = 8). Values indicate the mean ± SD.

eratively by the same procedure used in the control group. All of the rabbits were housed individually in metabolism cages and allowed to feed and drink ad libitum.

The heparinized samples of blood were collected by puncturing the marginal ear vein and immediately separating the plasma fraction. The samples of 24-hour urine and plasma were stored at −20° C until analyzed.

Intrauterine implantation of indomethacin. Implants (7 mm long and 3 mm in diameter) were prepared by mixing indomethacin powder with paraffin wax (melting point, 42° to 44° C) (rabbit rectal temperature: 39.3 ± 0.3° C; n = 7) in the ratio of 1 : 1 parts by weight.

On Day 9, an incision 5 mm long was made in the middle region of each uterine horn in the three rabbits already made pseudopregnant under intravenous pentobarbital anesthesia, and three or four implants containing a total of 100 mg of indomethacin were inserted. The incision in the horn was sutured with fine catgut. The rabbits were injected with tetracycline twice a day for 5 days postoperatively.

Analytical methods. Progesterone was measured by the radioimmunoassay method of MacDonald and associates.[18] (The antiserum to progesterone-3-carboxymethyloxime-bovine serum albumin was supplied by Dr. Kambegawa, of Teikoku Zoki Co., Ltd.) $PGF_{2\alpha}UM$ was assayed by the method of Ohki and associates.[19] Determination of $dhkPGF_{2\alpha}$ was carried out by the radioimmunoassay method of Satoh and associates.[17] Briefly, samples of plasma (2 to 4 ml) acidified to pH 3.0 were extracted with ethyl acetate. The $dhkPGF_{2\alpha}$ fraction separated by silicic acid column chromatography was subjected to radioimmunoassay. The assay solvent blank value was 0.04 ± 0.02 ng (n = 5). The recovery rates of $dhkPGF_{2\alpha}$ added (0, 0.5, 1.0, 2.0 ng) to 2 ml of plasma of the untreated rabbit were 0.22 ± 0.04 ng (n = 5), 0.67 ± 0.08 ng (n = 4) (91%), 1.10 ± 0.06 ng (n = 4) (88%), and 2.02 ± 0.12 ng (n = 4) (90%), respectively. The intra-assay coefficient of variation (CV) in six replicate determinations from a plasma pool was 9.5%. The corresponding interassay CV was 10.2% (n = 10). The limit of sensitivity of the assay was 30 pg. Since the antiserum used (supplied by Dr. Kirton, of The Upjohn Co., Ltd.) does not differentiate between $dhkPGF_{1\alpha}$ and $dhkPGF_{2\alpha}$, the values of $dhkPGF_{2\alpha}$ should be considered to be dhkPGF equivalents.

Results

Pseudopregnant rabbit. The life span of corpora lutea of eight pseudopregnant rabbits was monitored by the progesterone levels in peripheral plasma (Fig. 1). The progesterone concentration was 1.53 ± 1.28 ng/ml (mean ± SD) on Day 0 and increased to the highest level of 12.55 ± 3.01 ng/ml on Day 10. Thereafter, it declined gradually and reached the low level of 1.17 ± 0.82 ng/ml on Day 18. There was a significant difference in progesterone concentrations between Days 17 (5.71 ± 3.12 ng/ml) and 18 ($p < 0.02$), but not between Days 0 and 18. The finding indicates that the period of pseudopregnancy lasted 17 days.

The daily excretion levels of $PGF_{2\alpha}UM$ were 4.78 ± 1.06 µg/24 hr (mean ± SD) from Days 0 to 14 (Fig. 1). Levels increased from Day 15 to Day 18 and reached a peak of 21.92 ± 5.28 µg/24 hr on Day 18. Thereafter, $PGF_{2\alpha}UM$ levels fell to a level of 6.81 ±3.78

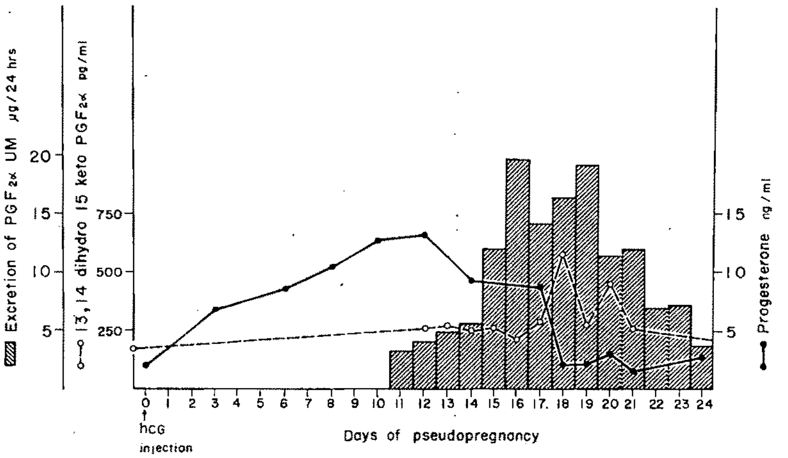

Fig. 2. Plasma level of progesterone and 13,14-dihydro-15-keto $PGF_{2\alpha}$ and 24-hour excretion of 5α,7α-dihydroxy-11-keto tetranorprostane 1,16-dioic acid ($PGF_{2\alpha}UM$) in urine during pseudopregnancy in rabbit A. Blood collection was performed at 0900 hours.

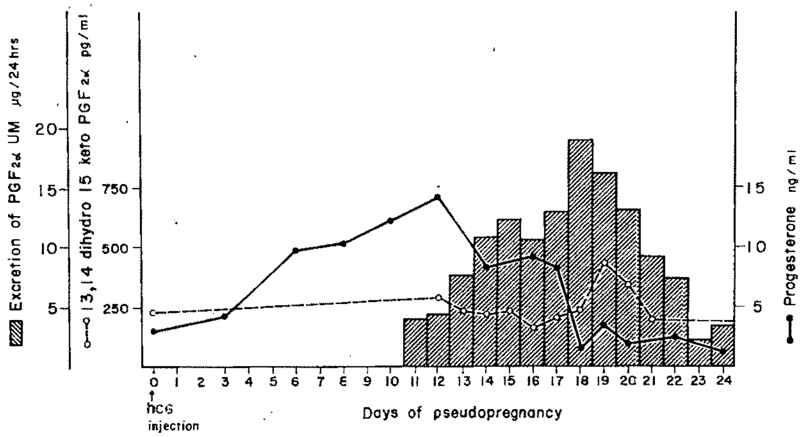

Fig. 3. Plasma level of progesterone and 13,14-dihydro-15-keto $PGF_{2\alpha}$ and 24-hour excretion of 5α,7α-dihydroxy-11-keto tetranorprostane 1,16-dioic acid ($PGF_{2\alpha}UM$) in urine during pseudopregnancy in rabbit B. Blood collection was performed at 0900 hours.

μg/24 hr on Day 21. $PGF_{2\alpha}UM$ excretion was highest from Days 15 to 20 ($p < 0.02$). Urine volumes ranged from 100 to 324 ml/24 hr (170.9 ± 59.23 ml/24 hr; n = 160 daily urine samples) in pseudopregnant rabbits. No changes in daily urine volumes were found at the time of luteal regression compared with those earlier.

The $dhkPGF_{2\alpha}$ concentration was measured during luteal regression in the peripheral blood of three pseudopregnant rabbits (timing: 0900 hours in rabbits A and B, at 0900 and 2100 hours in rabbit C). Duration of pseudopregnancy in these rabbits was 17 days as indicated by progesterone levels (Figs. 2, 3, and 4). In rabbit A, peaks in $dhkPGF_{2\alpha}$ concentration were observed on Days 18 (0.58 ng/ml) and 20 (0.45 ng/ml), but levels of approximately 0.25 ng/ml were seen on other days (Fig. 2).

High levels of $dhkPGF_{2\alpha}$ were detected for rabbit B on Days 19 (0.42 ng/ml) and 20 (0.34 ng/ml); otherwise the level was about 0.22 ng/ml (Fig. 3).

In rabbit C, moving elevations of $dhkPGF_{2\alpha}$ occurred on Days 18 (0.3, 0.16 ng/ml) and 19 (0.54, 0.28 ng/ml), with no large increase on other days (Fig. 4). Marked increases in $PGF_{2\alpha}UM$ excretion were evident in rabbits A, B, and C at about the time of luteal regression. A high level of $dhkPGF_{2\alpha}$ is considered to be related to a high excretion of $PGF_{2\alpha}UM$, but not the reverse.

Hysterectomized rabbit. Fig. 5 shows the mean level of progesterone and $PGF_{2\alpha}UM$ in six hysterectomized pseudopregnant rabbits. The progesterone concentration increased from 0.71 ± 0.33 ng/ml (mean ± SD) on Day 0 to 10.7 ± 4.21 ng/ml on Day 10, and thereafter decreased gradually to 0.75 ± 0.52 ng/ml on Day 27.

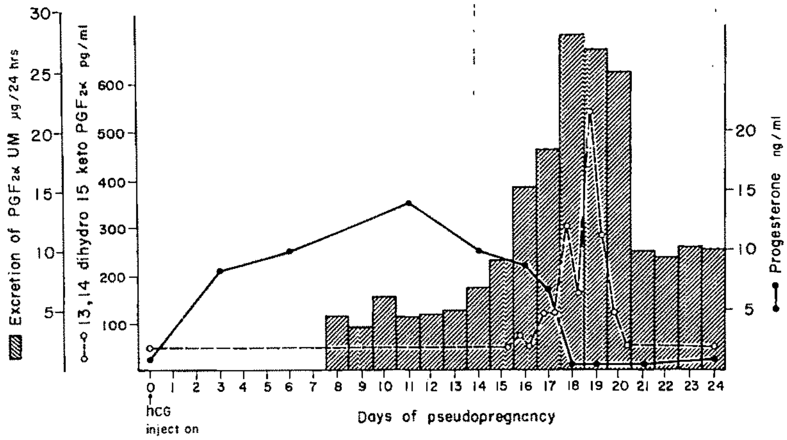

Fig. 4. Plasma level of progesterone and 13,14-dihydro-15-keto $PGF_{2\alpha}$ and 24-hour excretion of $5\alpha,7\alpha$-dihydroxy-11-keto tetranorprostane 1,16-dioic acid ($PGF_{2\alpha}UM$) in urine during pseudopregnancy in rabbit C. Blood collection was performed at 0900 and 2100 hours.

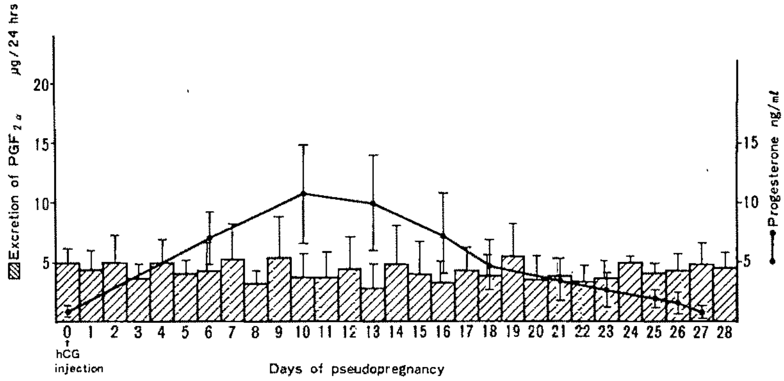

Fig 5. Plasma progesterone and 24-hour urinary excretion of $5\alpha,7\alpha$-dihydroxy-11-keto tetranorprostane 1,16-dioic acid ($PGF_{2\alpha}UM$) in hysterectomized pseudopregnant rabbits (n = 6). Values indicate the mean ± SD.

There was no significant difference in the progesterone levels on Day 10 between nonhysterectomized and hysterectomized pseudopregnant rabbits. This indicates that hysterectomy did not affect the ability of the pseudopregnant rabbit to produce progesterone at the peak of luteal function. The progesterone level of 1.83 ± 0.44 ng/ml on Day 25 was significantly higher than that on Day 0 ($P < 0.01$), whereas no significant difference was found between Days 0 and 26 (1.58 ± 0.97 ng/ml). Furthermore, the progesterone concentration on Days 18 (4.63 ± 2.07 ng/ml), 21 (3.37 ± 1.86 ng/ml), 23 (2.67 ± 1.69 ng/ml), and 25 in hysterectomized pseudopregnant rabbits was significantly higher ($P < 0.02$) than on Days 18, 19 (1.10 ± 0.86 ng/ml), and 22 (1.15 ± 0.8 ng/ml) in pseudopregnant rabbits. These findings indicate that the duration of pseudopregnancy in hysterectomized rabbits was 25 days, and that hysterectomy prolonged the life span of corpora lutea by 8 days.

$PGF_{2\alpha}UM$ excretion was almost constant from Day 0 to Day 28 at a level of 4.15 ± 1.83 µg/24 hours (n = 111 daily samples of urine), with a range of 3.15 to 5.58 ng/ml, thus indicating that no increase in the production of $PGF_{2\alpha}$ occurred during pseudopregnancy of the hysterectomized rabbit.

Intrauterine implantation of indomethacin. The plasma progesterone concentration and the daily $PGF_{2\alpha}UM$ excretion were determined in the three pseudopregnant rabbits bearing the indomethacin implants (rabbits D, E, and F). Duration of the life span of corpora lutea was 17 days in the four control pseudopregnant rabbits bearing the paraffin-wax implants alone.

The progesterone concentrations in rabbits D and E on Days 18 (3.8, 4.0 ng/ml), 19 (3.6, 3.7 ng/ml), 20 (2.6, 3.2 ng/ml), and 21 (2.3, 2.8 ng/ml) were higher than those on Day 0 (1.5, 1.7 ng/ml). Therefore, the life span of corpora lutea was prolonged 5 days by the ad-

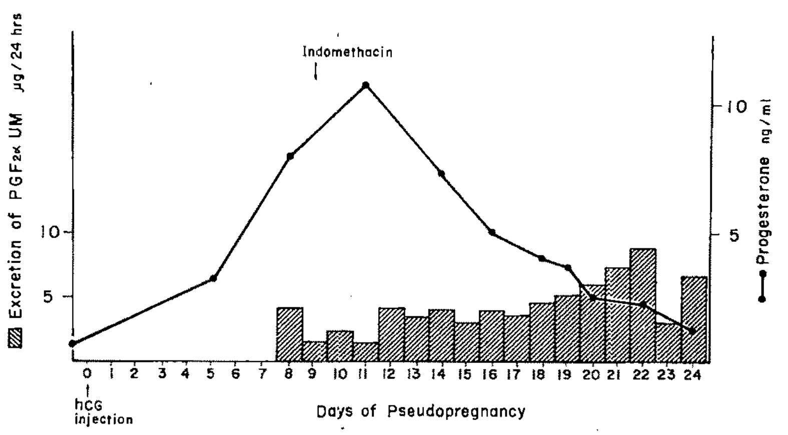

Fig. 6. Prolongation of pseudopregnancy by intrauterine indomethacin in rabbit D.

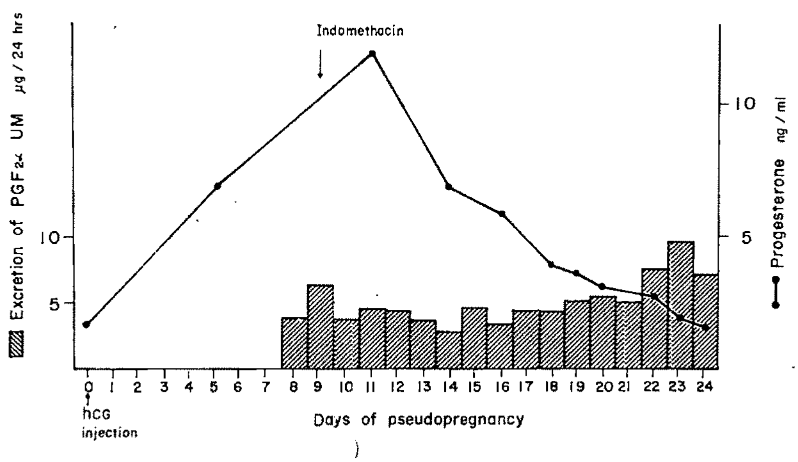

Fig. 7. Prolongation of pseudopregnancy by intrauterine indomethacin in rabbit E.

ministration of indomethacin in rabbits D and E. On the other hand, excretion levels of $PGF_{2\alpha}$ were low, ranging from 1.5 to 4.0 μg/24 hr (Figs. 6 and 7).

In the case of rabbit F (Fig. 8), the progesterone level dropped on Day 20 to 1.8 ng/ml (which was similar to the level of 1.6 ng/ml on Day 0) from 3.8 ng/ml on Day 18 and 3.6 ng/ml on Day 19. On the other hand $PGF_{2\alpha}UM$ began to increase from Day 20 (9.3 μg/24 hr) to Day 22 (22 μg/24 hr), with a corresponding decrease in progesterone. The survival period of corpora lutea was prolonged by only 2 days in rabbit F. The three pseudopregnant rabbits bearing the indomethacin implants were killed on Day 25, and the size of the implants was found to be half normal size.

Comment

In the present study, a marked increase in the production of $PGF_{2\alpha}$ occurred in the pseudopregnant rabbit in association with luteal regression as estimated by $PGF_{2\alpha}UM$ and $dhkPGF_{2\alpha}$. $PGF_{2\alpha}$ in the rabbit was reported to be excreted into urine in the form of six urinary metabolites, including $PGF_{2\alpha}UM$.[21] It has not been shown yet whether $PGF_{2\alpha}UM$ is the main urinary metabolite in the rabbit; however, the present findings indicate that $PGF_{2\alpha}UM$ excretion can be an indicator for monitoring $PGF_{2\alpha}$ production in the rabbit, although pulsatile production and release of $PGF_{2\alpha}$ are not detectable. The increase in $PGF_{2\alpha}$ production in the pseudopregnant rabbit was shown to start on Day 15, 3 days prior to luteolysis, and to reach the highest level on Day 18, 1 day after luteolysis. A similar excretory pattern for the main urinary $PGF_{2\alpha}$ metabolite was demonstrated in the guinea pig by Granström and Kindahl,[16] although the exact relationship between progesterone and the urinary $PGF_{2\alpha}$ metabolite was not clarified because of a lack of data on progesterone concentration.

Kindahl and associates[20] collected peripheral blood every hour for measurement of $dhkPGF_{2\alpha}$ and thereby demonstrated an intermittent release of large amounts

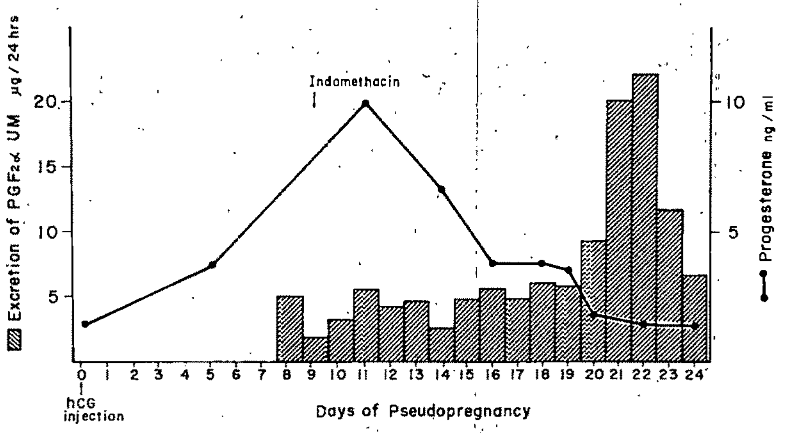

Fig. 8. Prolongation of pseudopregnancy by intrauterine indomethacin in rabbit F.

of $PGF_{2\alpha}$ during the last days of the estrous cycle of the ewe and heifer, and a concomitant decrease in progesterone.

The need for at least 5 ml of peripheral blood for the measurement of $dhkPGF_{2\alpha}$ in our assay system restricted us to collecting blood from the rabbit no more than twice a day. Therefore, any intermittent elevation of $dhkPGF_{2\alpha}$ could not be shown. An increase in $dhkPGF_{2\alpha}$ concentration in peripheral blood was found to occur concomitantly with luteal regression in the pseudopregnant rabbit.

The life span of corpora lutea was found to be prolonged by 8 days in hysterectomized pseudopregnant rabbits.[22, 23] Furthermore, no increase in $PGF_{2\alpha}UM$ excretion was found to be associated with the longer survival of corpora lutea. There has been no report concerning the correlation between hysterectomy and $PGF_{2\alpha}$ excretion in the pseudopregnant rabbit.

The present study showed that indomethacin, an inhibitor of PG biosynthesis, prolonged the life span of corpora lutea in the pseudopregnant rabbit. Indomethacin was demonstrated to have almost the same effect as hysterectomy on the corpora lutea in two rabbits, whereas in the third rabbit a 2-day prolongation of the life span was observed with an increase in $PGF_{2\alpha}UM$ excretion concomitant with luteolysis. This may be evidence explaining the action of $PGF_{2\alpha}$ as a uterine luteolysin. Horton and Poyser[24] reported that the intrauterine administration of indomethacin extended the length of the estrous cycle as much as fourfold in the guinea pig. According to the present data, indomethacin is not so effective in the pseudopregnant rabbit. On the other hand, there is a report that exogenous $PGF_{2\alpha}$ destroyed the corpora lutea in the hysterectomized gilt.[25] As mentioned above, there have been many reports of the relationship between $PGF_{2\alpha}$ and a uterine luteolysin. The present study provided confirmatory evidence that $PGF_{2\alpha}$ is a uterine luteolysin in the pseudopregnant rabbit, as it is in other species.[11-14]

We wish to thank Dr. K. Kirton, of The Upjohn Company, for supplying the antiserum to $dhkPGF_{2\alpha}$, Mr. F. Hirata, of Ono Pharmaceutical Company, for providing the antiserum to $PGF_{2\alpha}UM$, and Dr. Kambegawa, of Teikoku Zoki Company, for the antiserum to progesterone. We are grateful for the excellent assistance of Misses M. Tsuchiya, A. Ozaki, and N. Muro. Appreciation is also extended to Dr. R. G. Carlson, of The Upjohn Company, for his help in writing the manuscript.

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Intrauterine irrigation with cefamandole nafate solution at cesarean section: A preliminary report

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The effectiveness of intrauterine irrigation with an antibiotic solution of cefamandole nafate in reducing the incidence of endometritis after cesarean section was studied in a prospective, double-blind fashion. Ninety patients who underwent cesarean section at Tripler Army Medical Center were divided into three equal groups. Each group received one of the following treatments at the time of operation: (1) intrauterine irrigation with the antibiotic solution, (2) irrigation with normal saline solution, or (3) no irrigation. The resulting incidences of endometritis were 0%, 26.7%, and 23.3%, respectively. Intrauterine irrigation with cefamandole nafate solution at the time of cesarean section significantly reduced the incidence of endometritis. (AM. J. OBSTET. GYNECOL. 138:755, 1980.)

A COMMON problem in obstetric practice is the high rate of febrile morbidity after cesarean section. This morbidity is most often due to endometritis. Recent reports have indicated an incidence of endometritis as high as 85% in high-risk patients.[1] A variety of factors contributes to the development of endometritis. During labor, as the cervix dilates and loses its mucus plug, the endometrial cavity and amnion become exposed to the vaginal bacterial flora. When rupture of the membranes occurs for prolonged periods of time prior to delivery, the risk of developing endometritis after both vaginal delivery and cesarean section is increased. Other factors that favor endometritis include the number of vaginal examinations, length of labor, and length of invasive fetal monitoring.[2] Clotted blood, interstitial hemorrhage, and tissue necrosis resulting from the surgical trauma, as well as involutional sloughing of decidual tissue, provide conditions that enhance bacterial growth.

The parenteral use of antibiotics prophylactically in cesarean section has reduced the incidence of endometritis.[3] Uterine contractions after delivery may reduce the efficacy of parenteral antibiotics by preventing access to the surgical site. The topical use of antibiotics may avoid this and other disadvantages.

The purpose of this report is to describe the technique and results of the prophylactic use of intrauterine irrigation with an antibiotic solution. To our knowledge, no similar study has been performed.

Material and methods

All patients who underwent cesarean section at Tripler Army Medical Center during a 3-month study period were invited to participate in the study. Patients were excluded from the study if they had a history of penicillin or cephalosporin allergy, recent use of antibiotics, or a known infectious process, or if they lacked informed consent. Pharmacy personnel assigned each patient to one of three groups by means of a table of random numbers. This was blind to both patients and physicians. The three groups, each containing 30 patients, were as follows: *Group A*, irrigation with 2 gm of cefamandole nafate in 800 ml of normal saline solution (treated group); *Group B*, irrigation with 800 ml of normal saline solution (irrigation control group); and *Group C*, no irrigation (nonirrigation control group).

A vitamin* solution was added to both of the irriga-

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The views expressed herein are those of the authors and do not necessarily reflect the views of the United States Army or the Department of Defense.

Received for publication April 23, 1980.

Accepted July 30, 1980.

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*Solu-B-Forte, 5 drops.

Table I. Risk factors for development of endometritis

	Treated	*Irrigation control*	*Nonirrigation control*
No. of patients with rupture of membranes >6 hr	11	8	7
No. of patients in labor	22	19	19
Average hours of labor	9.0	10.5	9.5
No. of patients with invasive monitoring	12	9	9
Average hours of monitoring	8.0	11.1	7.8
Average no. of pelvic examinations in patients in labor	5.7	6.1	5.2

Table II. Risk factors versus endometritis

	Treated	*Irrigation control*	*Nonirrigation control*
Patients with rupture of membranes >6 hr:	11	8	7
No. developing endometritis (%)	0 (0)	4 (50.0)	5 (71.4)
Patients in labor:	22	19	19
No. developing endometritis (%)	0 (0)	8 (42.1)	7 (36.8)
Patients with invasive monitoring:	12	9	9
No. developing endometritis (%)	0 (0)	5 (55.5)	5 (55.5)

tion solutions in order to make them visually identical.

At the time of operation, after delivery of the infant, placenta, and membranes, the uterus was delivered through the abdominal incision. If the patient was in either the treated or irrigation control group, intrauterine irrigation was performed. A bulb syringe was used, with careful attention taken to ensure that the margins of the uterine incision received a direct application, and the irrigation was performed with some force so as to create a jetlike effect. The irrigant was simultaneously suctioned. Then, the uterine incision was repaired in a routine fashion, with additional irrigant applied to the closed incision and bladder flap. Finally, the uterus was replaced in the abdomen and, with the patient in reverse Trendelenburg position, the abdominal gutters were irrigated and suctioned. In the nonirrigation control group, the peritoneal gutters were cleared of clot and fluid by sponge or suction alone. Closure of the abdominal incision was performed in a fashion chosen by the surgeon, and any solution remaining was used to irrigate the wound during closure.

All procedures were performed by the resident and staff physicians on call at the time of the operation. Postoperative evaluations were made by those physicians who were usually assigned to that service. The physicians who were responsible for postoperative management did not know to which study group the patients had been assigned. Patients considered to have endometritis were those who developed a febrile course and whose physical and laboratory evaluations were consistent with the clinical diagnosis. Each of these patients was subsequently treated with parenteral antibiotics.

Results

The incidence of endometritis was 0 of 30 (0%) in the treated group, 8 of 30 (26.7%) in the irrigation control group, and 7 of 30 (23.3%) in the nonirrigation control group. By chi-square analysis, the difference in the rate of infection between the treated group and the average rate of infection of the control groups was statistically significant ($Z = -4.03$, $P < 0.01$).*

Table I compares preoperative risk factors and demonstrates an equal distribution among groups.

In Table II, risk factors are compared to the number of patients who developed endometritis. The treated group did have a slightly larger number of patients in the high-risk group (i.e., those with ruptured membranes over 6 hours, those in labor, and those with invasive fetal monitoring).

Table III compares the indications for cesarean section with the number of patients who developed endometritis. Those patients who developed endometritis are listed in Table IV, with the preoperative risk factors and their treatment group.

One patient in the treated group was placed on oral ampicillin for asymptomatic bacteriuria. No adverse consequences were noticed with the antibiotic irrigation. There were no long-term complications or late infections in the treated group.

In the irrigation control group, a total of 11 patients required antibiotics during their hospitalizations. Three patients with infection of the urinary tract were treated with oral antibiotic therapy. Eight patients with endometritis were treated parenterally with two or more drugs. One additional patient in the irrigation control group who developed a wound infection after

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*To obtain an estimate of this difference in rates, 0.5 was added to each cell in the 3×2 table. The Z statistic was then calculated on this adjusted count.

Table III. Indication for cesarean section versus endometritis

	Treated	*Irrigation control*	*Nonirrigation control*
Failure to progress (cephalopelvic disproportion):	10	11	7
No. with endometritis (%)	0 (0)	5 (45.5)	3 (42.8)
Repeat cesarean section:	11	13	12
No. with endometritis (%)	0 (0)	1 (7.7)	0 (0)
Breech malpresentations, twins, compound presentation:	9	6	11
No. with endometritis (%)	0 (0)	2 (33.3)	4 (36.4)

Table IV. Patients with endometritis

Indication	*Hour of ruptured membranes*	*Hours of labor*	*Hours of invasive monitoring*	*No. of examinations*	*Group*
Breech	17	4	0	1	Nonirrigation control
FTP*	15	13	10	13	Irrigation control
Twins	4.5	3	2	3	Nonirrigation control
FTP	12	12	12	6	Nonirrigation control
Repeat	0	7	0	2	Irrigation control
Breech	1	9	0	1	Irrigation control
FTP	20	20	16	12	Irrigation control
FTP	5	11	5	8	Irrigation control
Breech	7	5.5	1.5	3	Nonirrigation control
FTP	12.7	23	9	13	Nonirrigation control
FTP	24.5	24.5	14	11	Irrigation control
FTP	20.5	25	20.5	13	Irrigation control
Breech	2	12	0	1	Nonirrigation control
Breech	0	1	0	3	Irrigation control
FTP	16	20	11	12	Nonirrigation control

*Failure to progress.

discharge was treated on an outpatient basis with local therapy.

In the nonirrigation control group, eight patients were treated with antibiotics, one with intravenous ampicillin for pyelonephritis and seven with two or more parenteral antibiotics for endometritis.

Comment

The problem of postoperative infection has resulted in many attempts to use antibiotics prophylactically.[3] Appropriate prophylaxis must include a system that delivers adequate tissue levels of antibiotic to the surgical site before or very near the time that contamination occurs. In recent reports, the prophylactic administration of parenteral antibiotics has been advocated in patients who are undergoing cesarean section. Some authors suggest the administration of parenteral antibiotics prior to operation, with continuation of treatment for varying lengths of time postoperatively. Others recommend that the antibiotics be given at the time of operation when the umbilical cord is clamped, thus preventing exposure of the fetus to the drug. Each of these approaches has proved to be beneficial in reducing postpartum endometritis, but each has disadvantages. An antibiotic given prior to delivery may mask a neonatal infectious process by exposing the unborn infant to the drug. If the antibiotic is given after the umbilical cord has been clamped, there may not be an adequate level at the surgical site because of hemostatic suturing and the decrease in perfusion with uterine contractions after delivery.

In this study, an alternate method of prophylaxis is presented. Topically administered antibiotics should supply adequate tissue levels at the surgical site while not exposing the infant to the drug. The findings here support the efficacy of this prophylactic regimen.

The choice of cefamandole nafate was based on its demonstrated broad-spectrum activity against organisms which commonly cause puerperal endometritis.[4] Cefamandole has a high tissue affinity with a relatively long half life. Others who have used the cephalosporins in local irrigation have observed no adverse effects.[5]

Tables I, II, and III demonstrate that the various study groups were well matched for surgical indications and risk factors for endometritis. As expected, those patients in the control groups who were at greatest risk most often developed endometritis (i.e., 71.4% of those with rupture of membranes more than 6 hours in the nonirrigation control group). Although the treated group had more patients with high-risk factors, none of them developed endometritis. This study shows that excellent results can be anticipated if an antibiotic irrigation is used in patients who are at risk of developing endometritis. No comment can be made in regard to the effectiveness of antibiotic irrigation for those patients who are undergoing elective repeat cesarean section.

In conclusion, the prophylactic use of cefamandole nafate irrigation at the time of cesarean section markedly decreased the incidence of endometritis when compared to treatment with saline solution irrigation or no irrigation in similar groups of patients.

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Effect of urinary bladder fullness on fundal height measurements

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Measurements of fundal height were obtained on 117 obstetric patients with a full and with an empty bladder. Statistical testing demonstrated a significant difference between the two measurements. A full urinary bladder can change fundal height measurements and thus be a source of false positive variations in the progression of fundal height. There is a need to establish a baseline (with the bladder empty) before measuring fundal height. (AM. J. OBSTET. GYNECOL. 138:759, 1980.)

FUNDAL height measurements are routinely used in the clinical practice of obstetrics both for estimating gestational age and assessing the progression of pregnancy. Because there is such a wide variation in fundal heights for a particular gestational age, the method is not reliable for dating.[1] The fundal height progressively increases throughout pregnancy and reflects normal growth of the gestational contents at the rate of approximately 4 to 5 cm per month. Alterations in the progressive increase in fundal height may indicate certain conditions,[2] such as intrauterine growth retardation, oligohydramnios, polyhydramnios, and multiple gestations. If any of these conditions is suspected, ultrasonography may be indicated.

Since the effect that a full urinary bladder has on lifting structures out of the pelvis is well known to the ultrasonographer, this study was carried out to determine whether a full bladder changes the height of the uterine fundus and thus produces false positive measurements of fundal height progression.

Methods and material

One hundred seventeen obstetric patients were studied, with a range of 13 to 36 weeks' gestation. Population distribution is given in Table I. Gestational age was determined by measurements of biparietal diameter. Excluded from the study were patients with multiple gestations and abnormal gestations and obese patients.

Each patient underwent a routine obstetric examination with the use of commercially available gray-scale equipment with a 2.25 or 3.5 MHz transducer. Longitudinal and transverse scans of the pregnant uterus, biparietal diameters, and body diameters at the level of the umbilical vein were obtained.

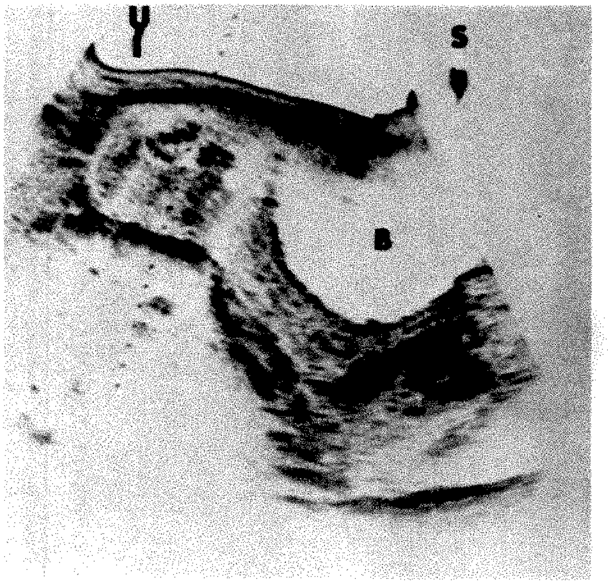

Fig. 1A. Longitudinal scan of pregnant uterus with full urinary bladder at 14 week's gestation. Fundal height was 18 cm. *U*, Umbilicus; *S*, pubic symphysis; *B*, bladder.

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Received for publication April 23, 1980.

Accepted July 30, 1980.

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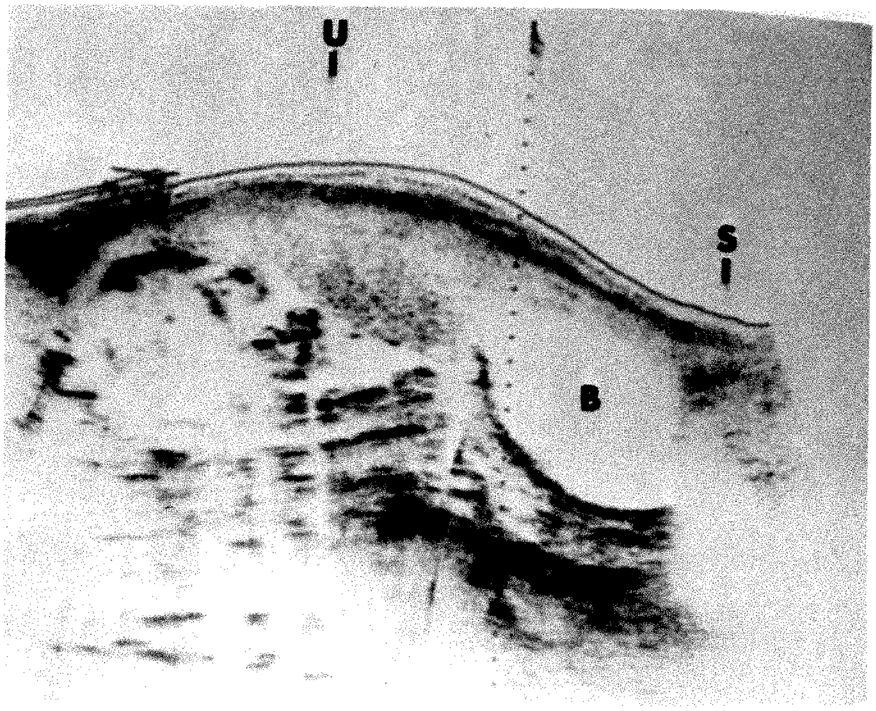

Fig. 1B. Longitudinal scan of pregnant uterus with full urinary bladder at 22 weeks' gestation. Fundal height was 26 cm. *U,* Umbilicus; *S,* pubic symphysis; *B, bladder.*

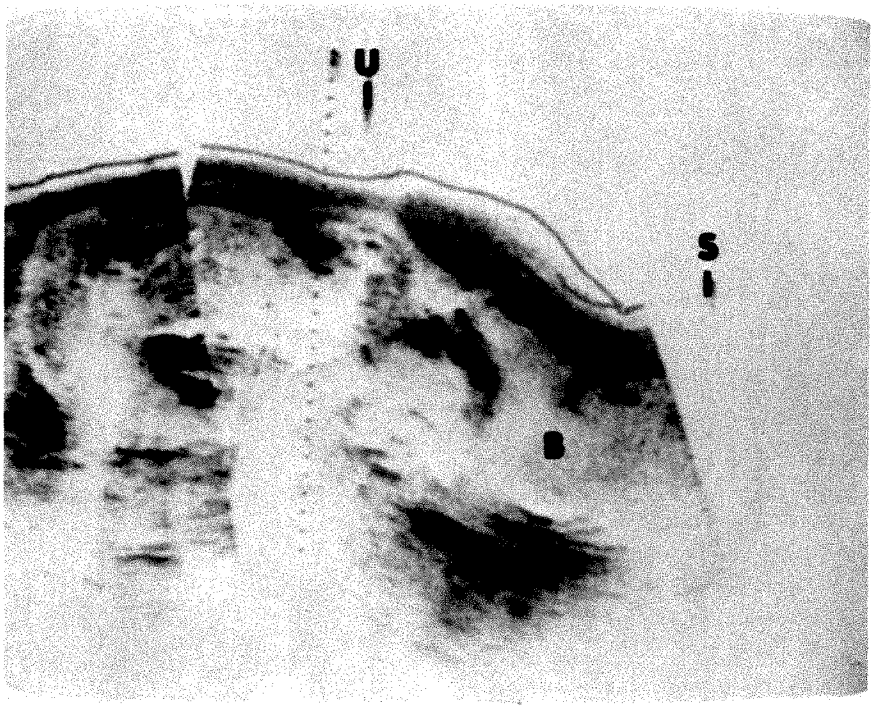

Fig. 1C. Longitudinal scan of pregnant uterus with full urinary bladder at 36 weeks' gestation. Fundal height was 40 cm. *U,* Umbilicus; *S,* pubic symphysis; *B,* bladder.

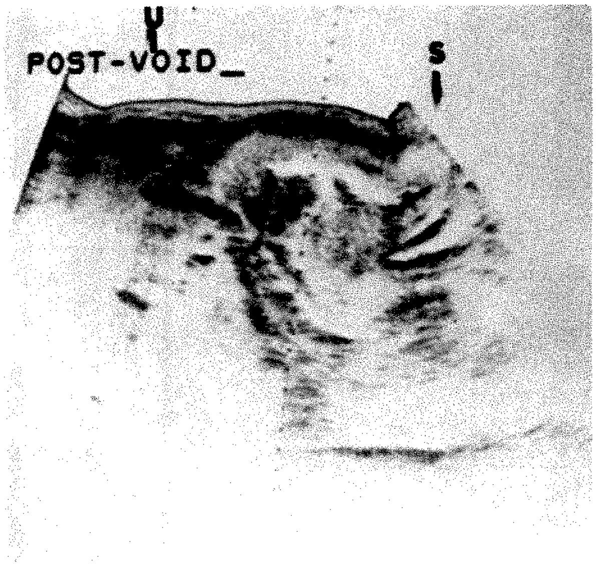

Fig. 2A. Longitudinal scan of same pregnancy as in Fig. 1A with empty urinary bladder. Fundal height was 14 cm.

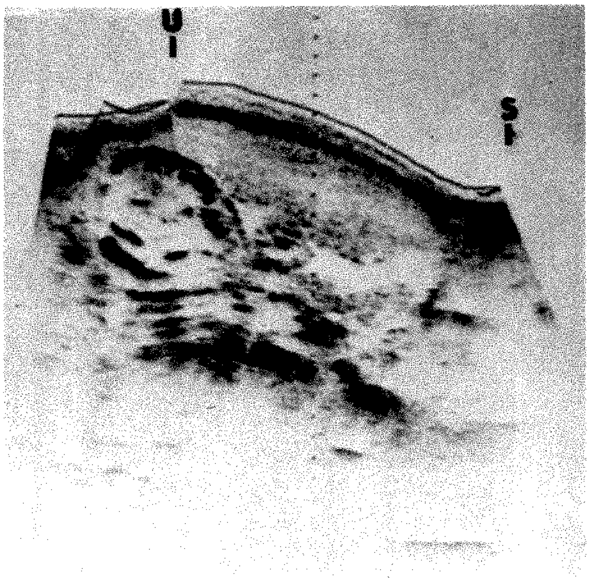

Fig. 2B. Longitudinal scan of same pregnancy as in Fig. 1B with empty urinary bladder. Fundal height was 22 cm.

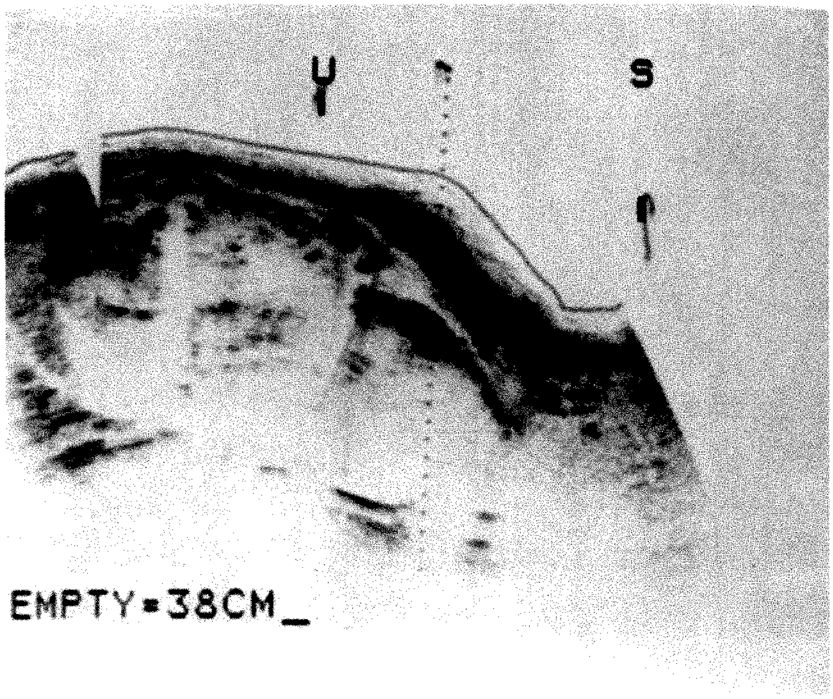

Fig. 2C. Longitudinal scan of same pregnancy as in Fig. 1C with empty urinary bladder. Fundal height was 38 cm.

Measurements of uterine fundal height were obtained with full and empty bladders. The pubic symphysis of each patient was marked to assure that measurements were obtained from the same origin, and the height of the uterine fundus was then checked by means of ultrasound guidance. Ultrasound was also used to make certain that the bladder was empty. Measurements were obtained with the use of a plastic centimeter tape, by two experienced technicians.

Data were grouped according to lunar months of gestation and statistically analyzed by Student's paired t test.

Table I. Distribution of patients and statistical analysis of fundal height measurements

Gestational age (wk)	*N*	*Mean F* ± SEM (cm)*	*Mean E† ± SEM (cm)*	*Mean difference, F-E‡*	*Range of F-E (cm)*
13-16	18	19.0 ± 3.3	16.4 ± 2.8	2.64 ± 0.38	0-7.0
17-20	24	22.4 ± 0.6	19.4 ± 0.5	3.10 ± 0.24	0-7.0
21-24	14	24.0 ± 0.8	22.0 ± 0.7	2.32 ± 0.41	0-5.0
25-28	17	29.1 ± 0.6	26.6 ± 0.5	2.71 ± 0.15	0-5.0
29-32	29	30.6 ± 0.5	28.2 ± 0.5	2.85 ± 0.23	0-6.0
33-36	15	33.7 ± 0.9	30.7 ± 0.9	2.90 ± 0.23	1-5.0

*F = Fundal height with full urinary bladder.
†E = Fundal height with empty urinary bladder.
‡All statistically significant at $P < 0.001$.

Results

Results are summarized in Table I. All groups demonstrated statistically significant differences at $P > 0.001$ in fundal heights between full and empty bladders. The average drop in fundal height ranged from 2.0 to 3.1 cm. The range of full minus empty (F − E) was 0 to 7 cm. Five patients showed no change in fundal height with filling of the bladder. Examples of variations in fundal height with a full and an empty bladder are shown in Figs. 1A to 1C and 2A to 2C.

Comment

Since fundal heights are useful in following the progression of pregnancy, there is a need for uniform measurements. This study showed that fundal height measurements are significantly altered by a full bladder. Therefore, a baseline is needed prior to obtaining fundal height measurements. Ideally, the patient should be measured immediately after voiding.

In the present study, plastic tapes were used in measuring fundal heights. Since calipers eliminate some of the abdominal curvature, they have been shown to give smaller numbers than plastic tapes when the same fundal heights are measured.[1] Statistically, variation in fundal heights with filling of the bladder would be even more significant with smaller numbers. Figs. 1A to 1C and 2A to 2C show that the measured change in fundal height with calipers would be greater than the results in the present study with plastic tapes. Thus, this change in fundal height secondary to bladder fullness may be more significant to the clinician who uses calipers for assessing his patients.

Since this study showed that bladder filling is a significant factor in fundal height measurements, and since a full bladder is a highly subjective and variable parameter, it seems to be necessary to establish a standard baseline for fundal height when the patient's bladder is empty. This is especially important in the high-risk obstetric patient, and in large clinics where the patient may have a long wait and is examined with varying degrees of bladder filling.

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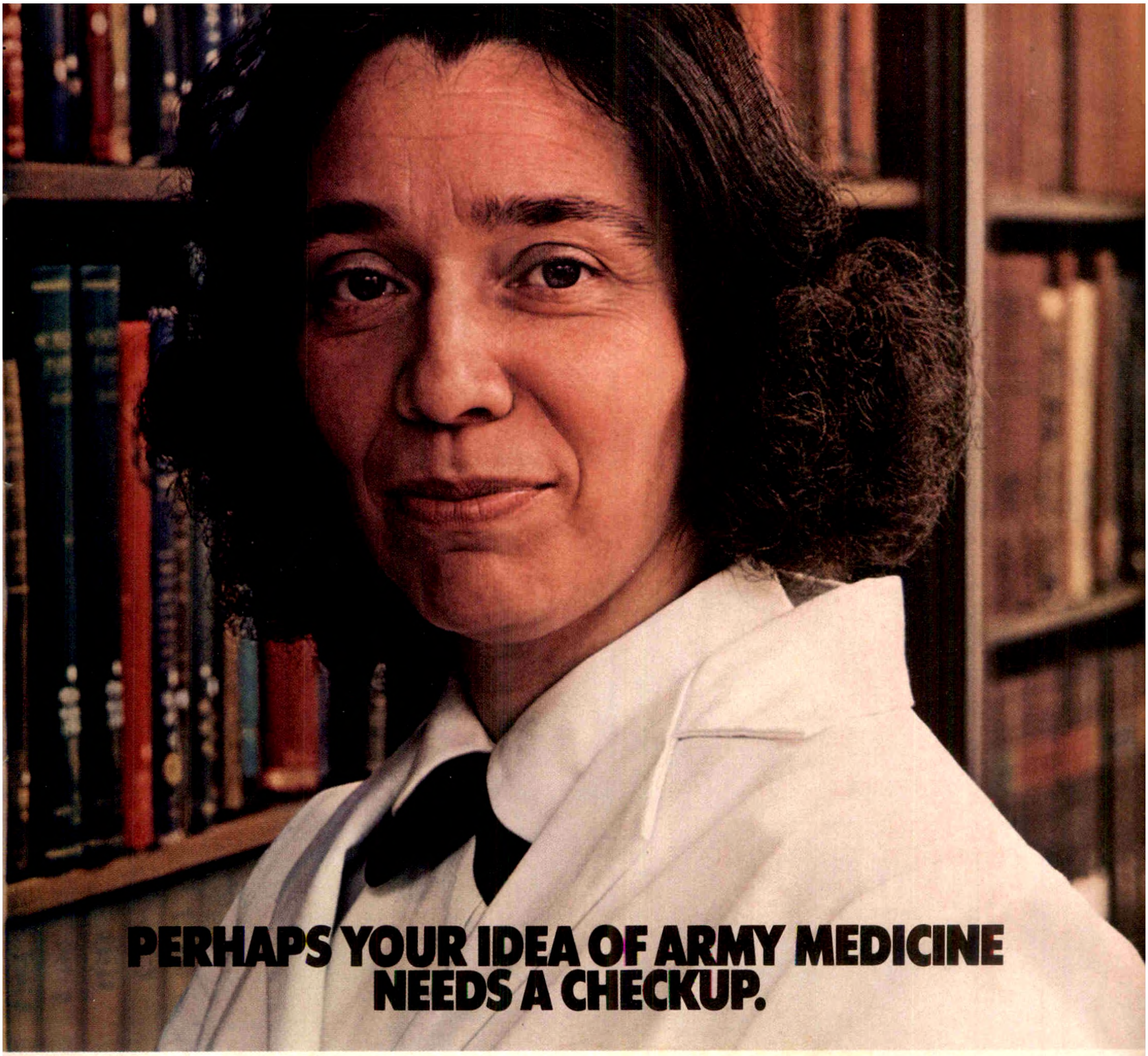

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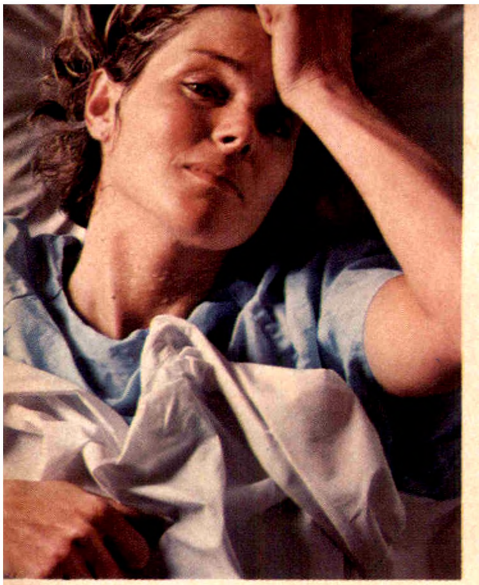

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0.3 mg/0.625 mg/1.25 mg/2.5 mg

FOR THE SHORT- AND LONG-TERM CONSEQUENCES OF SURGICAL MENOPAUSE

---

*Conjugated Estrogens Tablets have been evaluated as probably effective for postmenopausal osteoporosis.

See last page of this advertisement for prescribing information.

LATE TERIOR FLA PAINT

BRIEF SUMMARY
*(FOR FULL PRESCRIBING INFORMATION
AND PATIENT INFORMATION, SEE PACKAGE CIRCULAR)*
PREMARIN® Brand of Conjugated Estrogens Tablets, U.S.P.

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.

Three independent case control studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year.[1-3] This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.[4] The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment[1] and on estrogen dose.[3] In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration;[3] it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a non-steroidal estrogen have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare.[5,6] This risk has been estimated as not greater than 4 per 1000 exposures.[7] Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis,[8-12] epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb reduction defects.[13-16] One case control study[16] estimated a 4.7-fold increased risk of limb reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 per 1000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (Conjugated Estrogens Tablets, U.S.P.) for oral administration contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters.

INDICATIONS: Based on a review of PREMARIN Tablets by the National Academy of Sciences — National Research Council and/or other information, FDA has classified the indications for use as follows:

Effective:

1. Moderate to severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms, and they should not be used to treat such conditions.)
2. Atrophic vaginitis.
3. Kraurosis vulvae.
4. Female hypogonadism.
5. Female castration.
6. Primary ovarian failure.
7. Breast cancer (for palliation only) in appropriately selected women and men with metastatic disease.
8. Prostatic carcinoma — palliative therapy of advanced disease.
9. Postpartum breast engorgement — Although estrogens have been widely used for the prevention of postpartum breast engorgement, controlled studies have demonstrated that the incidence of significant painful engorgement in patients not receiving such hormonal therapy is low and usually responsive to appropriate analgesic or other supportive therapy. Consequently, the benefit to be derived from estrogen therapy for this indication must be carefully weighed against the potential increased risk of puerperal thromboembolism associated with the use of large doses of estrogens.[19]

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

"Probably" effective: For estrogen deficiency-induced osteoporosis, and only when used in conjunction with other important therapeutic measures such as diet, calcium, physiotherapy, and good general health-promoting measures. Final classification of this indication requires further investigation.

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (See Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast,[17] although a recent study has raised this possibility.[18] There is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.[17]

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement.[19-22] Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction.[23-30] Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users.[31,32] An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives.[33,34] If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown[35] to increase the risk of nonfatal myocardial infarction, pulmonary embolism and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives.[36] Increased blood pressure may occur with use of estrogens in the menopause[37] and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolau smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Oral contraceptives appear to be associated with an increased incidence of mental depression.[23a] Patients with a history of depression should be carefully observed. Preexisting uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not complete.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X, decreased antithrombin 3, increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome, amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis, change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION: 1. *Given cyclically for short term use only.* For treatment of moderate to severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 to 1.25 mg or more daily).

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (*e.g.*, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three to six month intervals.

2. *Given cyclically:* Female hypogonadism. Female castration. Primary ovarian failure. Osteoporosis.

Female hypogonadism — 2.5 to 7.5 mg daily, in divided doses for 20 days, followed by a rest period of 10 days' duration. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. The number of courses of estrogen therapy necessary to produce bleeding may vary depending on the responsiveness of the endometrium.

If bleeding occurs before the end of the 10 day period, begin a 20 day estrogen-progestin cyclic regimen with PREMARIN (Conjugated Estrogens Tablets, U.S.P.), 2.5 to 7.5 mg daily in divided doses, for 20 days. During the last five days of estrogen therapy, give an oral progestin. If bleeding occurs before this regimen is concluded, therapy is discontinued and may be resumed on the fifth day of bleeding.

Female castration and primary ovarian failure — 1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis (to retard progression) — 1.25 mg daily, cyclically.

3. *Given for a few days:* Prevention of postpartum breast engorgement — 3.75 mg every four hours for five doses, or 1.25 mg every four hours for five days.

4. *Given chronically:* Inoperable progressing prostatic cancer — 1.25 to 2.5 mg three times daily.

Inoperable progressing breast cancer in appropriately selected men and postmenopausal women — 10 mg three times daily for a period of at least three months.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

HOW SUPPLIED: PREMARIN (Conjugated Estrogens Tablets, U.S.P.) No. 865 — Each *purple* tablet contains 2.5 mg in bottles of 100 and 1,000. No. 866 — Each *yellow* tablet contains 1.25 mg in bottles of 100 and 1,000. Also in unit dose package of 100. No. 867 — Each *red* tablet contains 0.625 mg in bottles of 100 and 1,000. Also in unit dose package of 100. No. 868 — Each *green* tablet contains 0.3 mg in bottles of 100 and 1,000.

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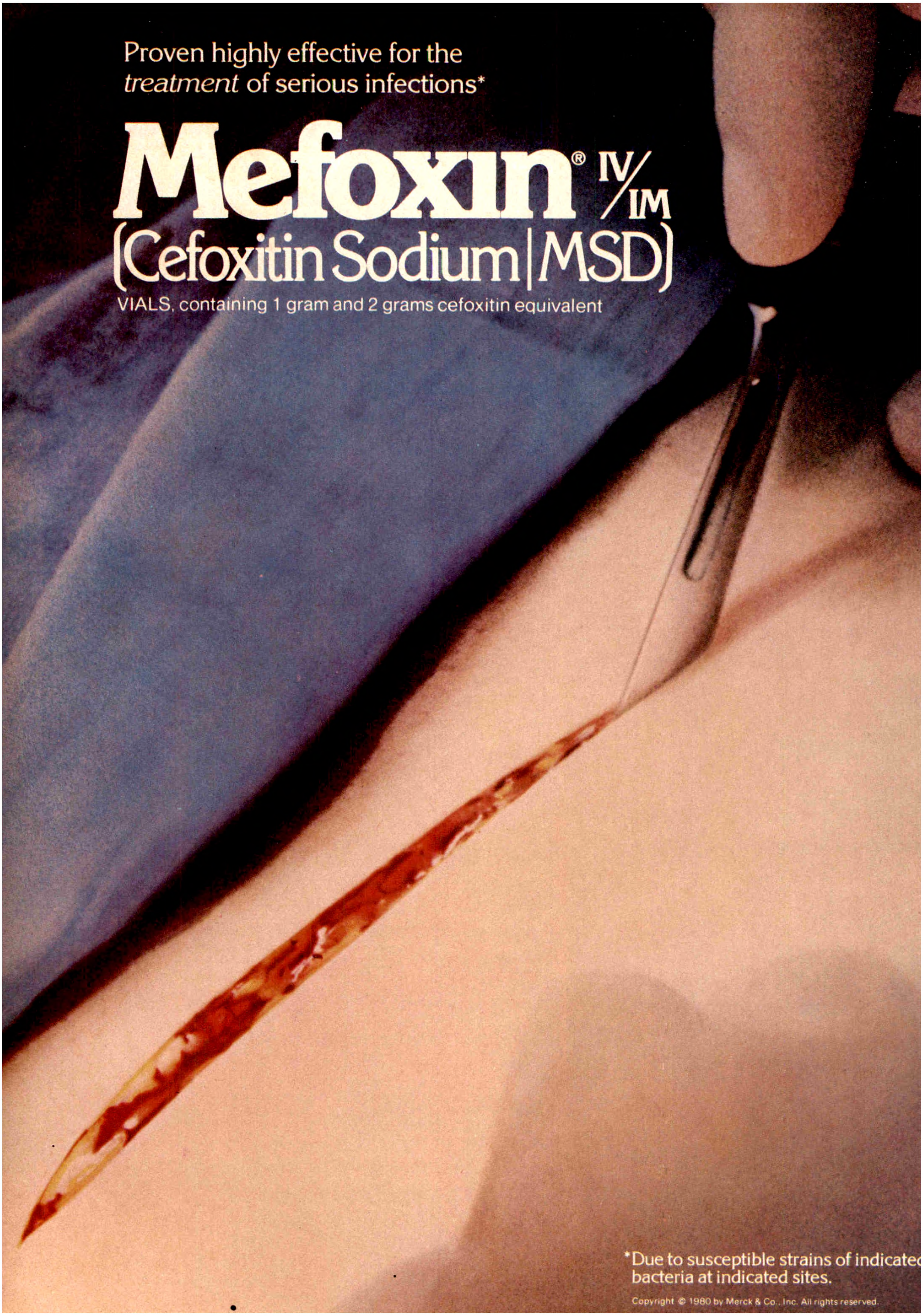

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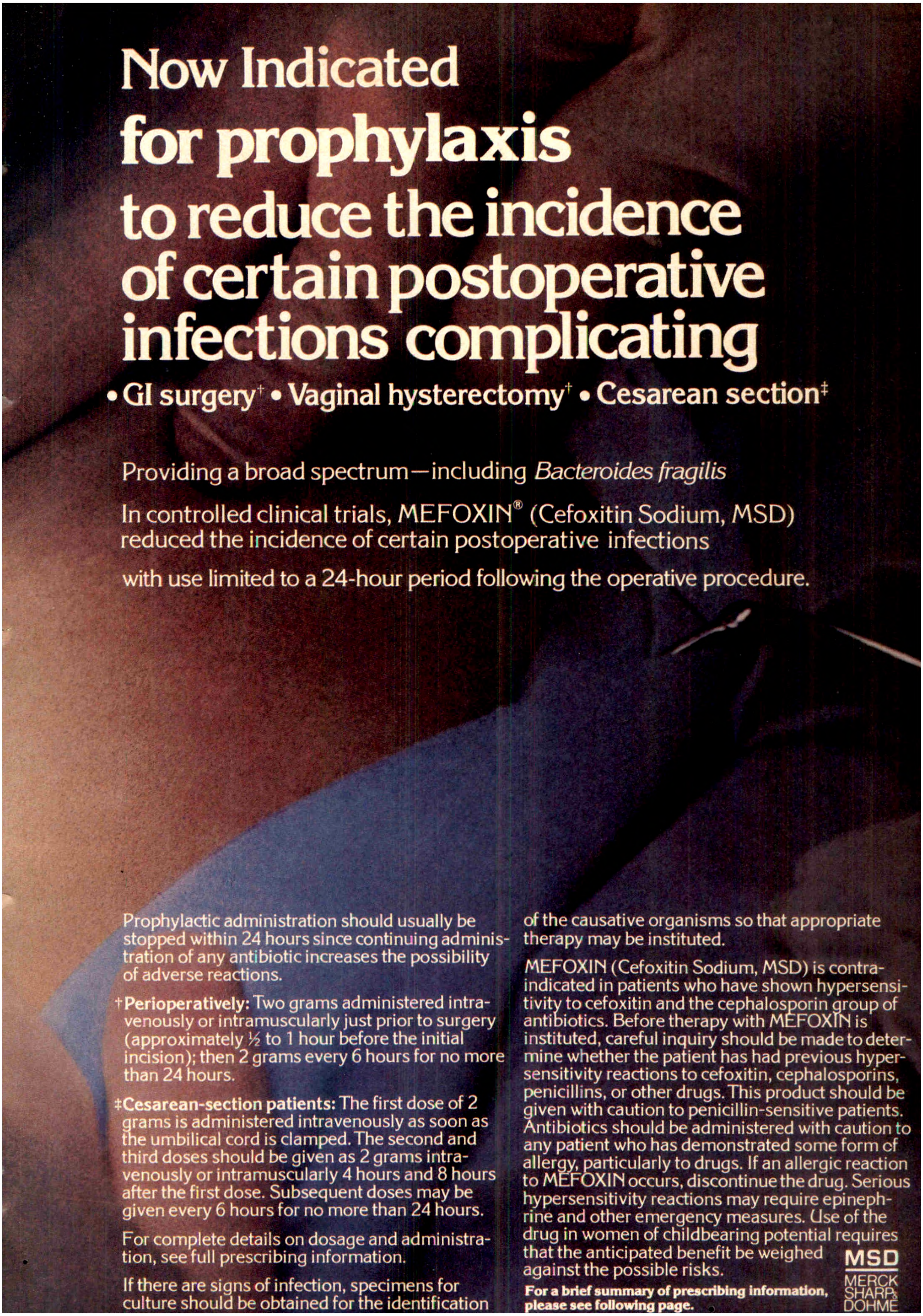

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‡**Cesarean-section patients:** The first dose of 2 grams is administered intravenously as soon as the umbilical cord is clamped. The second and third doses should be given as 2 grams intravenously or intramuscularly 4 hours and 8 hours after the first dose. Subsequent doses may be given every 6 hours for no more than 24 hours.

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If there are signs of infection, specimens for culture should be obtained for the identification of the causative organisms so that appropriate therapy may be instituted.

MEFOXIN (Cefoxitin Sodium, MSD) is contraindicated in patients who have shown hypersensitivity to cefoxitin and the cephalosporin group of antibiotics. Before therapy with MEFOXIN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefoxitin, cephalosporins, penicillins, or other drugs. This product should be given with caution to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to MEFOXIN occurs, discontinue the drug. Serious hypersensitivity reactions may require epinephrine and other emergency measures. Use of the drug in women of childbearing potential requires that the anticipated benefit be weighed against the possible risks.

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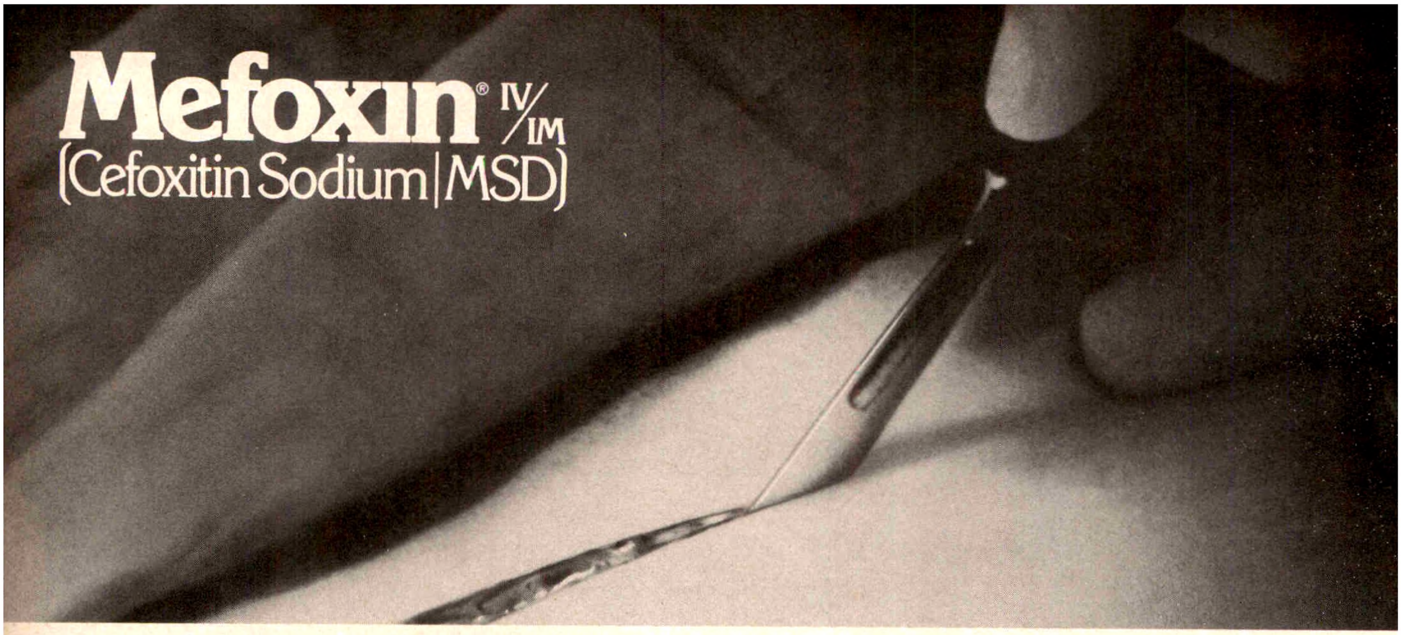

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GENITOURINARY INFECTIONS. Urinary tract infections caused by *E. coli*, *Klebsiella* species, *Proteus mirabilis*, indole-positive *Proteus* (i.e., *P. morganii*, *P. rettgeri*, and *P. vulgaris*), and *Providencia* species. Uncomplicated gonorrhea due to *Neisseria gonorrhoeae*.

INTRA-ABDOMINAL INFECTIONS, including peritonitis and intra-abdominal abscess, caused by *E. coli*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,‡ and *Clostridium* species.

GYNECOLOGICAL INFECTIONS, including endometritis, pelvic cellulitis, and pelvic inflammatory disease, caused by *E. coli*, *N. gonorrhoeae*, *Bacteroides* species including the *B. fragilis* group,‡ *Clostridium* species, *Peptococcus* species, *Peptostreptococcus* species, and group B streptococci.

SEPTICEMIA caused by *Strep. pneumoniae* (formerly *D. pneumoniae*), *Staph. aureus* (penicillinase and non-penicillinase producing), *E. coli*, *Klebsiella* species, and *Bacteroides* species including the *B. fragilis* group.‡

BONE AND JOINT INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing).

SKIN AND SKIN STRUCTURE INFECTIONS caused by *Staph. aureus* (penicillinase and non-penicillinase producing), *Staph. epidermidis*, streptococci (excluding enterococci, e.g., *Strep. faecalis*), *E. coli*, *P. mirabilis*, *Klebsiella* species, *Bacteroides* species including the *B. fragilis* group,‡ *Clostridium* species, *Peptococcus* species, and *Peptostreptococcus* species.

Although appropriate culture and susceptibility studies should be performed, therapy may be started while awaiting these results. Cefoxitin is not active *in vitro* against most strains of *Pseudomonas aeruginosa* and enterococci (e.g., *Strep. faecalis*) and many strains of *Enterobacter cloacae*. Methicillin-resistant staphylococci are almost uniformly resistant to cefoxitin.

Prevention– Prophylactic use perioperatively (preoperatively, intraoperatively, and postoperatively) in surgical procedures (e.g., vaginal hysterectomy, gastrointestinal surgery) classified as contaminated or potentially contaminated or in patients in whom infection at the operative site would present a serious risk, e.g., prosthetic arthroplasty; intraoperatively (after umbilical cord is clamped) and postoperatively in cesarean section.

MEFOXIN usually should be given ½ to 1 hour before the operation, which is sufficient time to achieve effective levels in the wound during the procedure. Prophylactic administration should usually be stopped within 24 hours since continuing administration of any antibiotic increases the possibility of adverse reactions but, in the majority of surgical procedures, does not reduce the incidence of subsequent infection. However, in patients undergoing prosthetic arthroplasty, it is recommended that MEFOXIN be continued for 72 hours after the surgical procedure. If there are signs of infection, specimens for culture should be obtained for the identification of the causative organism so that appropriate therapy may be instituted.

Contraindications: Previous hypersensitivity to cefoxitin and the cephalosporin group of antibiotics.

Warnings: BEFORE THERAPY IS INSTITUTED, CAREFUL INQUIRY SHOULD BE MADE TO DETERMINE PREVIOUS HYPERSENSITIVITY REACTIONS TO CEFOXITIN, CEPHALOSPORINS, PENICILLINS, OR OTHER DRUGS. GIVE WITH CAUTION TO PENICILLIN-SENSITIVE PATIENTS. ANTIBIOTICS SHOULD BE ADMINISTERED WITH CAUTION TO ANY PATIENT WHO HAS DEMONSTRATED SOME FORM OF ALLERGY, PARTICULARLY TO DRUGS. IF AN ALLERGIC REACTION TO CEFOXITIN OCCURS, DISCONTINUE THE DRUG. SERIOUS HYPERSENSITIVITY REACTIONS MAY REQUIRE EPINEPHRINE AND OTHER EMERGENCY MEASURES.

Precautions: The total daily dose should be reduced in patients with transient or persistent reduction of urinary output due to renal insufficiency because high and prolonged serum antibiotic concentrations can occur in such individuals from usual doses. As with other antibiotics, prolonged use may result in overgrowth of nonsusceptible organisms; repeated evaluation of the patient's condition is essential. If superinfection occurs, take appropriate measures. Increased nephrotoxicity has been reported following concomitant administration of cephalosporins and aminoglycoside antibiotics.

Interference with Laboratory Tests—As with cephalothin, high concentrations (>100 mcg/ml) may interfere with measurement of serum and urine creatinine levels by the Jaffé reaction and produce false increases of modest degree in creatinine levels reported; serum samples should not be analyzed for creatinine if withdrawn within 2 hours of cefoxitin administration. A false-positive reaction for glucose in urine has been observed with CLINITEST§ reagent tablets.

Pregnancy—In women of childbearing potential, weigh anticipated benefit against possible risks.

Nursing Mothers—Cefoxitin is excreted in human milk in low concentrations.

Infants and Children—Safety and efficacy in infants from birth to three months have not yet been established. In children three months and older, higher doses have been associated with increased incidence of eosinophilia and elevated SGOT.

Adverse Reactions: The most common adverse reactions have been local reactions following intravenous or intramuscular injection. Other adverse reactions have been encountered infrequently. *Local Reactions*—Thrombophlebitis with intravenous administration; pain, induration, and tenderness after intramuscular injections. *Allergic Reactions*—Rash, pruritus, eosinophilia, fever, and other allergic reactions. *Gastrointestinal*—Nausea, vomiting, and diarrhea. *Blood*—Transient eosinophilia, leukopenia, neutropenia, and hemolytic anemia; a positive direct Coombs test may develop in some individuals, especially those with azotemia. *Liver Function*—Transient elevations in SGOT, SGPT, serum LDH, and serum alkaline phosphatase. *Renal Function*—Elevations in serum creatinine and/or blood urea nitrogen levels.

Note: In group A beta-hemolytic streptococcal infections, therapy should be maintained for at least 10 days to guard against the risk of rheumatic fever or glomerulonephritis. In staphylococcal and other infections involving a collection of pus, surgical drainage should be carried out where indicated. Intramuscular injections should be well within the body of a relatively large muscle such as the upper outer quadrant of the buttock (i.e., gluteus maximus); aspiration is necessary to avoid inadvertent injection into a blood vessel. The total daily dosage in infants and children should not exceed 12 grams.

How Supplied: Sterile cefoxitin sodium in vials and infusion bottles containing 1 gram or 2 grams cefoxitin equivalent.

For more detailed information, consult your MSD representative or see full prescribing information. Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, PA 19486

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‡*B. fragilis, B. distasonis, B. ovatus, B. thetaiotaomicron, B. vulgatus*

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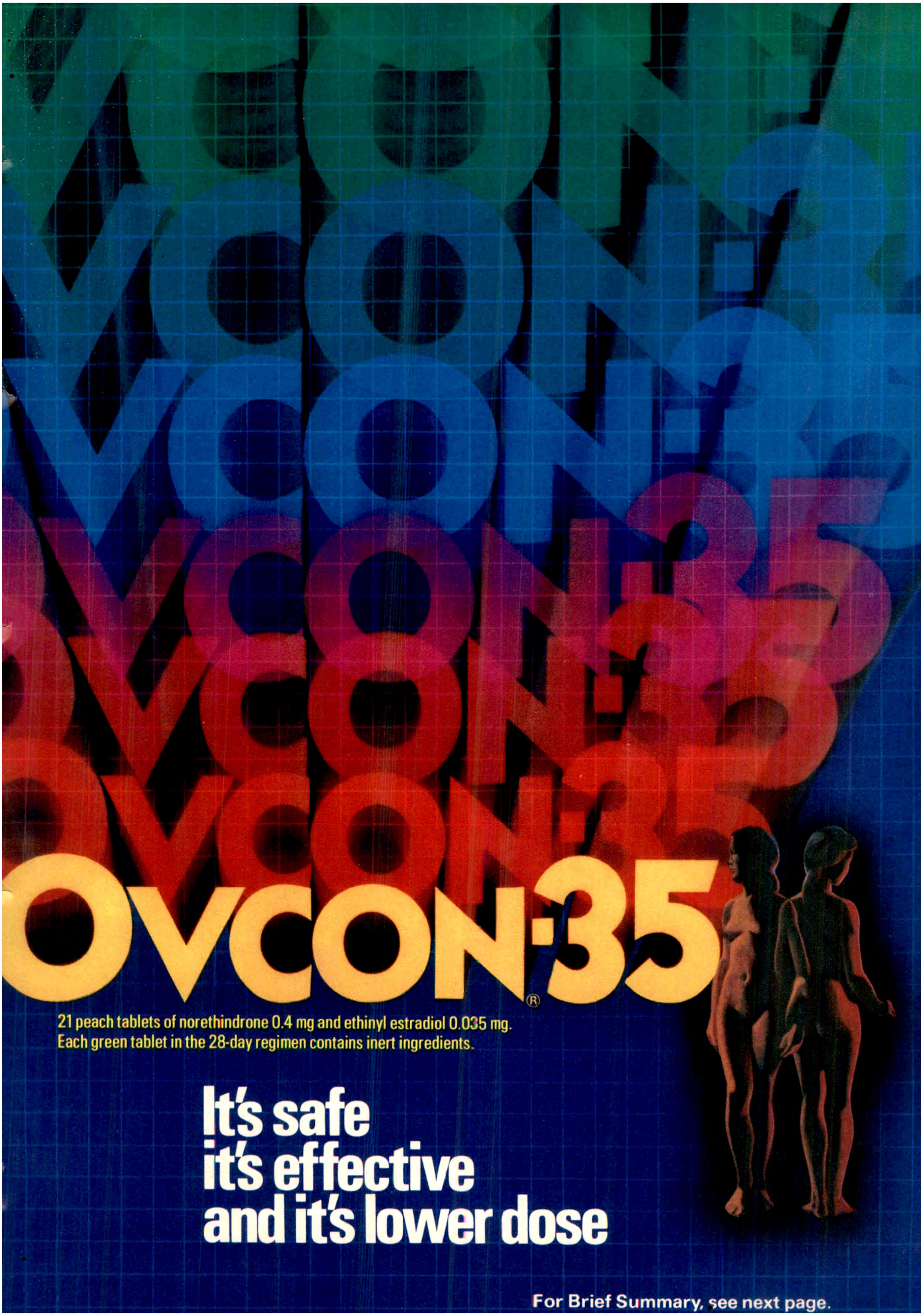

21 peach tablets of norethindrone 0.4 mg and ethinyl estradiol 0.035 mg.
Each green tablet in the 28-day regimen contains inert ingredients.

For Brief Summary, see next page.

OVCON®-35

21 peach tablets of norethindrone 0.4 mg and ethinyl estradiol 0.035 mg. Each green tablet in the 28-day regimen contains inert ingredients.

Indications and Usage: Ovcon is indicated for the prevention of pregnancy. Some combination products containing 35 mcg. or less of ethinyl estradiol report slightly higher pregnancy rates than are reported for the higher dose combination products.

Contraindications: Oral contraceptives should not be used in women with any of the following conditions: 1) Thrombophlebitis or thromboembolic disorders. 2) A past history of deep vein thrombophlebitis or thromboembolic disorders. 3) Cerebral vascular or coronary artery disease. 4) Known or suspected carcinoma of the breast. 5) Known or suspected estrogen dependent neoplasia. 6) Undiagnosed abnormal genital bleeding. 7) Known or suspected pregnancy.

Warnings:

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. Women who use oral contraceptives should be strongly advised not to smoke. Use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1) *Thromboembolic Disorders and Other Vascular Problems.* An increased risk of thromboembolic and thrombotic disease associated with use of oral contraceptives is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of oral contraceptives are 4 to 11 times more likely than nonusers to develop these diseases without evident cause. In a study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater. An increased risk of myocardial infarction associated with the use of oral contraceptives has been reported. Studies conducted in the United Kingdom found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that oral contraceptive users who do not smoke (smoking considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction compared to nonusers who do not smoke. Oral contraceptive users who smoke have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. The amount of smoking is a very important factor. British investigators concluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; preparations containing 100 mcg. or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg. of estrogen. The relative risk of thromboembolic disease associated with progestin-only oral contraceptives has not been determined. Cases of thromboembolic disease have been reported in women using progestin-only products, and they should not be presumed to be risk-free. The overall excess mortality rate annually from circulatory diseases for oral contraceptive users was estimated to be 20 per 100,000 (ages 15-35, 5/100,000; ages 35-44, 33/100,000; ages 45-49, 140/100,000), the risk being concentrated in older women, in those with a long duration of use, and in cigarette smokers. The highest risk was found in heavy cigarette smokers (15 or more cigarettes per day) who used oral contraceptives and were aged 40 or older. Women who smoke should be advised not to use oral contraceptives. The use of oral contraceptives in women over age 40 with other risk factors is not recommended. The mortality associated with all of the methods of birth control is low compared to the risk of childbirth, with the exception of oral contraceptive users who smoke and are over age 40. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with oral contraceptives increases with age after approximately age 30 and for myocardial infarction is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. If feasible, oral contraceptives should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization. 2) *Ocular Lesions.* There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with the use of oral contraceptives. Discontinue the medication if there is unexplained sudden or gradual, partial or complete loss of vision; sudden onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3) *Carcinoma.* Long term administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. In humans, one study investigated the first 21 cases of endometrial adenocarcinoma in women on oral contraceptives reported to a registry. Of those women without predisposing risk factors for this disease, nearly all occurred in women who had used a sequential oral contraceptive. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestin-only oral contraceptives. No increase in breast cancer in women taking oral contraceptives has been reported although one study reported an increased risk of breast cancer in subgroups of women treated using oral contraceptives with documented benign breast disease. There is at present no confirmed evidence from human studies of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of all women taking oral contraceptives is essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use oral contraceptives. 4) *Hepatic Adenoma.* Benign hepatic adenomas have been found to be associated with the use of oral contraceptives. One study reported a higher risk associated with oral contraceptive formulations with high hormonal potency. Although benign and rare, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short term as well as long term users of oral contraceptives. Two studies relate the risk with duration of contraceptive use, the risk being much greater after 4 or more years of oral contraceptive use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock. A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time. 5) *Use in Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.* Fetal abnormalities have been reported to occur in the offspring of women who have taken progestogens and/or estrogens during pregnancy. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. A high percentage of such exposed women (30% to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Similar data are not available with the use of other estrogens but it cannot be presumed that they would not induce similar changes. The data suggest that the risk of limb reduction defects in exposed fetuses is somewhat less than 1 in 1000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications and there is no evidence from well-controlled studies that progestins are effective for these uses. Increases in chromosomal aberrations have been reported in women who become pregnant soon after ceasing oral contraceptive therapy. Embryos with these anomalies are virtually always spontaneously aborted. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping oral contraceptives is unknown. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing the contraceptive regimen. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed the patient should be apprised of the potential risks to the fetus and the advisability of pregnancy continuation should be discussed in light of these risks. It is also recommended that women who discontinue oral contraceptives with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend three months. Administration of progestin-only or progestin-estrogen combinations to induce withdrawal bleeding should not be used as a test for pregnancy. 6) *Gall Bladder Disease.* Studies have reported an increased risk of surgically confirmed gallbladder disease appearing after one year of oral contraceptive use and doubling of the risk after 4 or 5 years of use. 7) *Carbohydrate and Lipid Metabolic Effects.* A decrease in glucose tolerance has been observed in a significant percentage of patients on oral contraceptives. For this reason, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives. Increased serum levels of triglycerides and total phospholipids have been observed in oral contraceptive users. The clinical significance of this observation is unknown at this time. 8) *Elevated Blood Pressure.* An increase in blood pressure has been reported in women receiving oral contraceptives. The prevalence of hypertension in oral contraceptive users may be no higher than nonusers in the first year of oral contraceptive use but increases with longer exposure and in the fifth year of use is two and one-half to three times the reported prevalence in the first year. Women who previously had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given oral contraceptives. 9) *Headaches.* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of oral contraceptives and investigation of the cause. 10) *Bleeding Irregularities.* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another formulation may solve the problem. Changing to an oral contraceptive with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. 11) *Ectopic Pregnancy.* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. However, in oral contraceptive failures, the ratio of ectopic to intrauterine pregnancies is higher than in women not using oral contraceptives since the drugs are more effective in preventing intrauterine rather than ectopic pregnancy. The higher ectopic-intrauterine ratio has been reported with both combination products and progestin-only oral contraceptives. 12) *Breast Feeding.* A small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs. The long-range effect to the nursing infant cannot be determined at this time.

Precautions: 1) A complete medical and family history should be taken prior to the initiation of oral contraceptives. Examination should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than 1 year without another physical examination being performed. 2) Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3) Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 4) Because oral contraceptives may cause some degree of fluid retention, conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, cardiac or renal insufficiency or asthma require careful observation. 5) Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving oral contraceptive therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6) Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7) Oral contraceptive users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8) Serum folate levels may be depressed by oral contraceptive therapy. This may complicate subsequent pregnancy with regard to folate deficiency. 9) The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted. 10) Certain endocrine and liver function tests and blood components may be affected by estrogen-containing oral contraceptives: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG, free T_4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient: Detailed Patient Labeling has been prepared for use by the patient and has been made available for distribution by the pharmacist.

Drug Interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium and ampicillin.

Adverse Reactions: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **Warnings**): Thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies. There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis; benign hepatomas; neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle (Other reactions, as a general rule, are seen much less frequently or only occasionally.); gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes (tenderness, enlargement, and secretion); change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis. The following adverse reactions have been reported in users of oral contraceptives, and the association has been neither confirmed nor refuted: Premenstrual-like syndrome, intolerance to contact lenses, change in corneal curvature (steepening), cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function.

Acute Overdosage: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

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PHARMACEUTICAL DIVISION

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Changes in glycosaminoglycan composition of normal human placentas with maturation

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Crude glycosaminoglycan preparations were isolated from acetone-powder of first-, second-, and third-trimester human placentas. Glycosaminoglycan composition was examined by electrophoresis and densitometric scanning before and after treatment with testicular hyaluronidase and chondroitinase ABC. Hexosamines were determined in electrophoretic fractions in order to identify the individual glycosaminoglycans. The results showed a decrease in glycosaminoglycans and an increase in protein contents in the preparations as the placenta matured. The main glycosaminoglycans in all placentas were dermatan sulfate, chondroitin sulfates, hyaluronic acid, and heparan sulfate. With aging of the placenta, the proportion of hyaluronic acid remained unchanged and that of chondroitin sulfate decreased, whereas the proportions of dermatan and heparan sulfates increased. Age-dependent changes in the glycosaminoglycan composition of the placenta appear to follow a pattern similar to that reported for other mammalian tissues.
(Am. J. Obstet. Gynecol. 138:763, 1980.)

THE PLACENTA is a highly vascularized organ and the physicochemical properties of the connective tissue components interposed between the maternal and fetal circulations might be of great importance in the regulation of the intensive exchange of materials. The study of the glycosaminoglycan (GAG) composition of the placenta is of considerable interest in view of the role played by these constituents of connective tissue in transport processes. Several works have been published on the content and characterization of human placental GAG,[1-3] but, possibly because of differences in analytical procedures, there is no complete agreement concerning the composition.

Furthermore, information on changes in the content of these substances occurring with maturation of the placenta is very scanty. We believe that investigation of these changes could be of importance when dealing with a short-lived organ undergoing a rapid development, in view of the widely reported quantitative and

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This paper is part of a thesis submitted by Lina Wasserman to Tel-Aviv University, in partial fulfillment of the requirements for the Ph.D. degree.

Received for publication March 26, 1980.

Accepted August 13, 1980.

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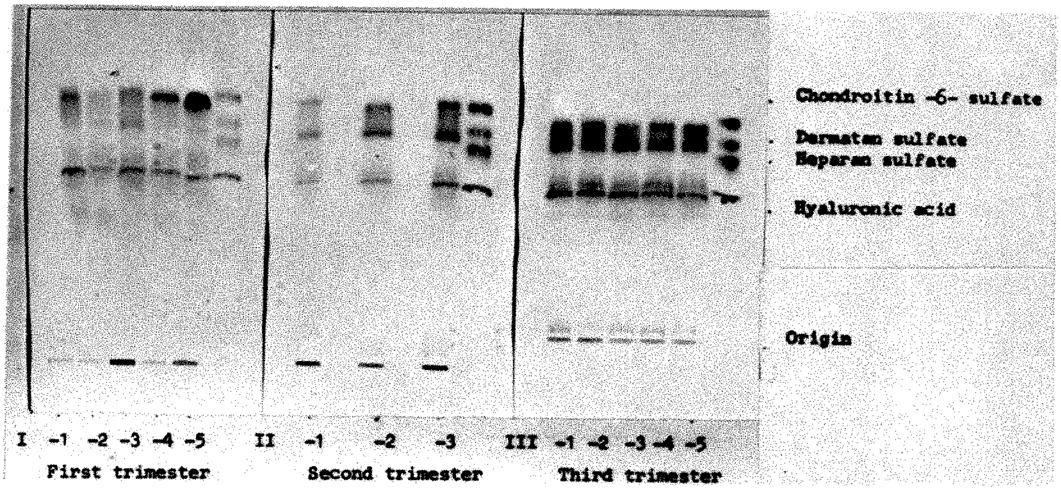

Fig. 1. Electrophoretic separation of GAG in crude preparations from placentas at three stages of development. The separation was carried out on cellulose acetate in 0.1M cadmium acetate buffer, pH 4.0, 0.8 mA/cm for 1 to 2 hours. Markers in the order of migration were HA, HS, DS, and CS, which appear at the right edge of each electrophoretogram. The figures at the bottom represent the number of the preparation (see Table I) in each trimester.

Table I. Yield and composition of crude GAG preparations from placentas in various stages of development

Trimester of pregnancy	*No. of placenta*	*Yield of crude GAG preparation in dry defatted tissue (%)*	*Uronic acid in GAG preparation (%)*	*Protein in GAG preparation (%)*	*Uronic acid in dry defatted tissue (%)*
I	1	4.22	20.4	7.8	0.86
	2	5.53	19.6	5.0	1.08
	3	3.84	13.7	5.0	0.53
	4	3.34	20.4	5.6	0.68
	5	2.65	16.7	12.3	0.44
	Mean ± SD	3.92 ± 1.07	18.2 ± 2.9	7.1 ± 3.1	0.72 ± 0.25
II	1	1.68	12.1	6.7	0.20
	2	1.52	14.6	7.3	0.22
	3	1.35	12.5	10.6	0.17
	Mean ± SD	1.52 ± 0.16	13.1 ± 1.3	8.2 ± 2.1	0.20 ± 0.02
III	1	1.74	9.6	20.0	0.17
	2	1.93	8.3	24.0	0.16
	3	1.68	12.5	15.0	0.21
	4	1.74	10.4	20.0	0.18
	5	1.38	10.8	17.0	0.15
	Mean ± SD	1.69 ± 0.20	10.3 ± 1.5	19.2 ± 3.4	0.17 ± 0.02

qualitative alterations taking place in GAG of various organs and biologic fluids with development and aging.[4-7]

The present work was designed to characterize the GAG of the normal human placenta at term and at two additional stages of development.

Material and methods

Five first-trimester and three second-trimester placentas collected after therapeutic abortions, and five normal term placentas obtained after spontaneous delivery were used. After being freed from the umbilical cord and the chorioallantoic membrane with the large blood vessels attached, the placentas were homogenized in acetone. After several daily changes of acetone, the tissue was defatted with petrol ether, dried in air, and ground to a fine powder.

The isolation of GAG was carried out according to the procedure of Curwen and Smith,[8] as follows. After heating in a boiling water bath for 20 minutes in order to inactivate hydrolytic enzymes, a suspension of the powder in 0.2M Tris buffer, pH 8.0, was treated with pronase (B grade, Calbiochem, 100 μg/mg of dry defatted tissue) at 37° C for 48 hours. An additional proteolytic digestion was carried out with crude papain (5 μg/mg of dry defatted tissue, Sigma Chemical Co.) in 0.1M phosphate buffer, pH 7.0, containing 0.01M cysteine and 0.01M ethylenediaminetetra-acetic acid, at

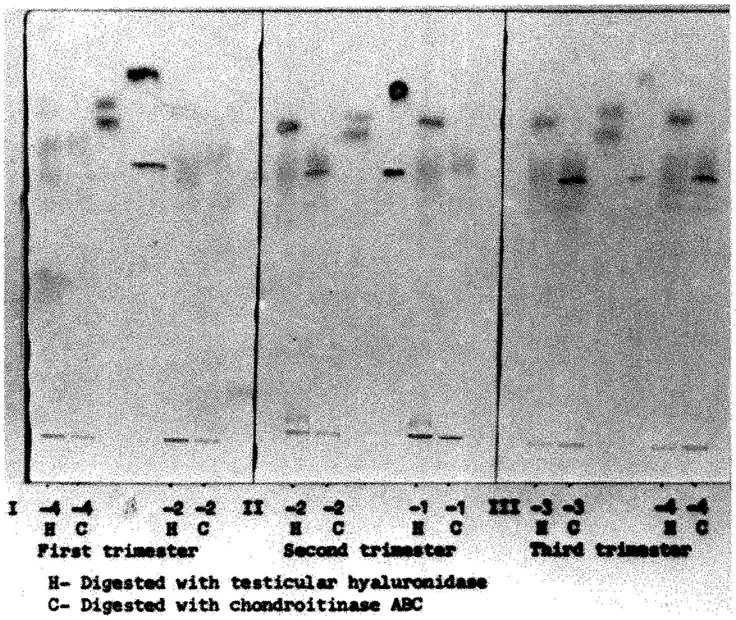

Fig. 2. Electrophoretic separation of GAG in preparations after degradation with testicular hyaluronidase *(H)* and chondroitinase ABC *(C)*. Markers in the order of migration were HS and DS (third path) and HA and CS (fourth path). Conditions and signs as in Fig. 1.

65° C for 24 hours. The digest was treated with trichloroacetic acid (final concentration, 5%) for 4 hours at 4° C. After centrifugation, the GAG were recovered from the supernatant by precipitation with three volumes of cold 5% potassium acetate in absolute ethanol. The precipitate was then washed successively with absolute ethanol, ethanol:ether (1:1), and, finally, ether. Nucleic acids were eliminated by incubation of the preparations at 37° C for 16 hours with deoxyribonuclease (25 μg/ml, Schwarz/Mann) and ribonuclease (10 μg/ml, Calbiochem) in 0.05M phosphate buffer, pH 7.0, containing 0.005M $MgCl_2$. After dialysis for 4 days against distilled water, the retentate was lyophilized. The residue was then dissolved in water, and the GAG were precipitated with three volumes of 5% potassium acetate in ethanol. The crude preparations obtained were weighed.

Uronic acid was determined according to the method of Bitter and Muir,[9] and protein by the method of Lowry and associates[10] with the use of bovine serum albumin (Nutritional Biochemicals) as standard.

Electrophoretic separation of GAG was carried out on cellulose acetate with the use of 0.1M cadmium acetate buffer, pH 4.0, and 0.8 mA/cm for 1 to 2 hours.[8] The individual fractions were located by staining with 0.2% alcian blue 8 GN (Matheson, Coleman & Bell) in ethanol: glacial acetic acid: water (100:50:850) and destained with 7.5% acetic acid. Quantitation of individual GAG was performed by densitometric scanning at 525 nm with the Helena autoscanner. Marker GAG were obtained from Dr. Cifonelli, University of Chicago.

For better identification of individual GAG the preparations were treated either with testicular hyaluronidase (Sigma), 70 U/mg in 0.1M acetate buffer–0.15M NaCl, pH 5.0, at 37° C for 24 hours, or with chondroitinase ABC (Seikagaku Kogyo Co), 0.2 U/mg in enriched Tris buffer at 37° C for 24 hours.[11] The undigested material, consisting of the unsusceptible GAG, was precipitated with ethanol–5% potassium acetate, separated by cellulose acetate electrophoresis as described above, and located with alcian blue. GAG from other electrophoresis strips were recovered, after staining with 0.05% toluidine blue in 80% ethanol, by destaining successively with 0.2M ammonium hydroxide in 80% ethanol and in 80% ethanol and by elution with water.[12] The eluate served for the identification of hexosamines made by paper chromatography[13] of hydrolyzates in 4N HCl at 100° C for 6 hours.

Results

The yields by weight of crude preparations, the concentrations of uronic acid (which is a measure of GAG content) and of protein, as well as the final recovery of uronic acid from the dry defatted tissue are presented in Table I. It can be seen that the yields of the preparations obtained from first-trimester placentas are considerably higher than those of preparations obtained from placentas in the other stages of development, and

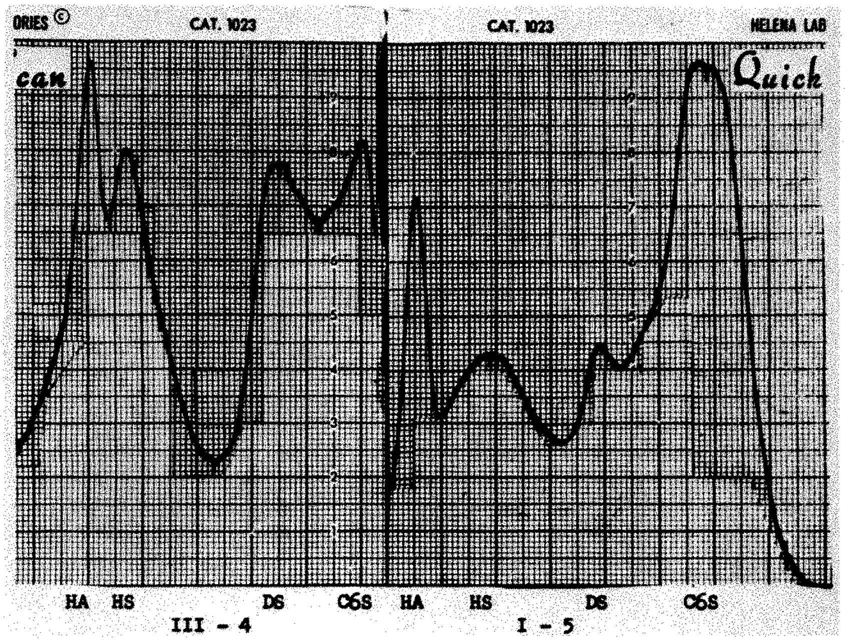

Fig. 3. Representative densitometric scannings of electrophoretograms from a term *(III-4)* placenta and a first-trimester *(I-5)* placenta.

Table II. Percentages of individual GAG in placentas at various stages of development

Trimester of pregnancy	*GAG*	*Percentage (Mean ± SD)*	*Comparison to term placenta*
I	Hyaluronic acid	24.4 ± 4.5	P = 0.05
	Heparan sulfate	12.1 ± 1.4	P = 0.005
	Dermatan sulfate	17.0 ± 5.1	P = 0.001
	Chondroitin 4/6 sulfate	46.5 ± 8.2	P = 0.001
II	Hyaluronic acid	19.3 ± 3.2	P = 0.5
	Heparan sulfate	10.0 ± 0.1	P = 0.001
	Dermatan sulfate	37.0 ± 2.6	P = 0.1
	Chondroitin 4/6 sulfate	33.7 ± 5.2	P = 0.25
III	Hyaluronic acid	20.3 ± 1.2	—
	Heparan sulfate	15.1 ± 1.0	—
	Dermatan sulfate	34.2 ± 2.5	—
	Chondroitin 4/6 sulfate	30.3 ± 2.5	—

that GAG concentrations decrease in the preparations as maturation progresses. These differences result in a clear decrease in GAG recovery from the dry defatted tissue, as seen in Table I. Of interest is the increase in protein concentration with maturation of the placenta which might result from a decreasing susceptibility to the proteinases used in the isolation procedure.

Fig. 1 presents the electrophoretograms of all the preparations examined. It can be seen that all yielded four bands, two of which (1 and 4) could be readily identified as hyaluronic acid (HA) and chondroitin 4/6 sulfate (C4/6S), respectively, by comparison with markers. Band 2 occupied a position very close to that of HA, and band 3 an intermediate one between those of dermatan sulfate (DS) and heparan sulfate (HS).

Additional experiments involving the combined use of enzymes and electrophoresis helped in the characterization of these fractions. To this aim, electrophoresis was performed of digests of the preparations after incubation with testicular hyaluronidase or chondroitinase ABC. As seen in Fig. 2, bands 2 and 3 consisting of GAG not susceptible to testicular hyaluronidase, corresponded to the two intermediate bands in Fig. 1, thus suggesting the possible presence of HS and DS. On the other hand, bands 1 and 4 disappeared, thus confirming their identity as HA and C4/6S. Treatment with chondroitinase ABC resulted in the complete disappearance of bands 3 and 4 confirming their identity as DS and C4/6S. Bands 1 and 2, however, remained unchanged, thus suggesting the presence of HS (band 2) and possibly HA, which has been shown[14] to be partially resistant to chondroitinase ABC under the conditions used.

Further support for the identity of bands 2 and 3 was provided by hexosamine analysis, which was carried out in eluates from electrophoretograms after digestion with testicular hyaluronidase, by means of paper chromatography. Thus, band 3 gave one spot corresponding to galactosamine, which is a constituent of DS. Band 2 showed a main spot corresponding to glucosamine, which points to the presence of HS, and a minor spot of galactosamine, which could originate from small amounts of partly degraded DS. It has been reported in this connection that placental DS is somewhat degradable by testicular hyaluronidase.[2]

From these experiments, the conclusion can be drawn that the main GAG of the placentas at all stages examined are DS, C4/6S, HA, and HS. The reason for lack of a thorough correspondence of electrophoretic mobilities with reference markers has not been elucidated but most probably can be attributed to the variations in molecular size and degree of sulfation characterizing this class of compounds.

The mean proportions of individual GAG in the preparations from the placentas of the three stages, obtained by scanning densitometry of electrophoretograms, performed in triplicate, are shown in Table II. It is seen that the proportion of HA, amounting to about 20%, does not change significantly with the development of placenta. HS was higher in term placenta than in the placenta of earlier stages. Of significance was the increase in the proportion of DS and the decrease in C4/6S that occurred after the first trimester. It is of interest that neither substance showed any marked differences in concentrations between the second and third trimesters.

The differences in the proportions of individual GAG between the youngest-state placenta and term placenta are illustrated in Fig. 3.

Comment

The present work shows that the main GAG of human placenta are DS, C4/6S, HA, and HS. In this respect, the results are in accordance with those of other works,[1, 2] although there is no complete agreement with regard to their proportions, which may be due to differences in analytical procedures.

Several changes in the total content and in composition of GAG have been found with maturation of the placenta. Thus, first-trimester placentas were shown to contain higher percentages of GAG in dry tissue than did older ones. In this respect, placental tissue follows the general pattern of age changes observed in other tissues.[4] It should be noted also that crude GAG preparations obtained from young placentas contained higher percentages of GAG and had a lower residual protein content after proteolytic digestions than did older ones. It is of interest in this connection that an increasing resistance of protein associated with GAG to proteolytic enzymes has been reported by us to occur with advancing age in GAG preparations from urine.[7] The proportion of HA appeared to remain practically unchanged with maturation, and that of HS increased slightly after the second trimester. The most prominent changes were observed in the proportions of DS and C4/6S, namely, DS increased and C4/6S decreased considerably after the first trimester. These results are in general agreement with the findings of Lee and associates,[2] except for the very slight changes in C4/6S reported by those authors. In contrast, Lovell and associates[3] failed to detect age-related changes except for an increase in the sulfation of C6S. Alterations in GAG distribution with advancing age in various organs have been widely reported,[4-7] although the nature of them may vary according to tissue. The general tendency appears to be an increase in HS and DS at the expense of HA and C4/6S, together with an increase in sulfation. In the placenta, however, the proportion of HA does not seem to decrease during its development. This might be related to the role played by this substance in permeability phenomena, as well as in the state of hydration and swelling capacity of the tissue,[15] which could be decisive in the prevention of compression of the placenta.[3] With regard to HS and DS, the increase in them with maturation might be related to the development of the villous tree blood vasculature, which has been reported to contain these compounds.[14]

It is interesting to point out that major changes in the GAG pattern occur in the first trimester and that no marked differences could be observed thereafter. This might be related to the fact that early in the second trimester the placenta completes its general differentiation and organization as a definite organ, and thereafter growth takes place primarily by the enlargement of fetal cotyledons formed previously.[16] A similar phenomenon has been observed in chicken skin.[4] Thus, the pattern of GAG in the skin of 4-week-old chickens was closer to that in the skin of 23-month-old chickens than to that in the skin of newly hatched chickens.

This work, performed on normal tissue, could serve as a basis for comparison with pathologic placentas and for a better understanding of the possible involvement of the GAG in the development of abnormal conditions.

We wish to thank Mrs. Y. Herzig for performing the densitometric scanning.

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Glycosaminoglycan patterns in diabetic and toxemic term placentas

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Crude glycosaminoglycans were prepared from acetone powder of diabetic, toxemic, and normal term placentas. Glycosaminoglycan composition was determined by electrophoresis and densitometric scanning with and without treatment with testicular hyaluronidase and chondroitinase ABC. The identity of individual glycosaminoglycans was confirmed by the nature of their hexosamine. Glycosaminoglycan content was found to be significantly increased in diabetic placentas and increased to a lesser degree in the toxemic placentas. The amount of hyaluronic acid was elevated in both abnormal tissues, and heparan sulfate was slightly higher in diabetes, while unchanged in toxemia. Dermatan sulfate was markedly reduced in the abnormal placentas while chondroitin 4/6 sulfate was unaltered. An attempt was made to correlate the histopathologic changes reported to occur in these conditions with the alterations in the glycosaminoglycans patterns of placentas. (AM. J. OBSTET. GYNECOL. 138:769, 1980.)

THE VASCULOSYNCYTIAL MEMBRANE of the placenta is very active in the transfer processes of this organ and the physicochemical properties of the components of its connective tissue may be involved in the regulation of these functions. The glycosaminoglycans (GAG) of normal human placenta have been characterized by us[1] and by others[2] and have been shown to vary in amount and in the proportion of the individual members of this class of compounds with maturation.

Moreover, changes in the consumption and distribution of GAG in tissues are known to occur in the course of development of pathologic conditions.[3] The present work was designed to determine total GAG content and proportions of individual GAG in placentas in two common pathologic conditions of pregnancy, namely, diabetes and toxemia, both characterized by changes in placental morphology. The aim of this work was to compare the GAG composition of these placentas to that of normal ones of the same gestational age in an attempt to establish a possible correlation between changes in these substances and the development of the pathologic conditions.

Material and methods

Five diabetic, three toxemic, and five normal term placentas were used. The tissue was freed from the umbilical cord, the chorioallantoic membrane, and the associated large blood vessels and was homogenized in acetone.

Following several changes of acetone and defatting with petrol ether, the placentas were ground to a fine powder. The isolation of a crude GAG preparation was carried out as previously reported.[1, 4] Briefly, the acetone powder was treated successively with pronase (B grade, Calbiochem) and with crude papain (Sigma Chemical Co.). After deproteinization with trichloroacetic acid the GAG were precipitated from the super-

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This paper is part of a thesis submitted by Lina Wasserman to Tel-Aviv University, in partial fulfillment of the requirements for the Ph.D. degree.

Received for publication March 26, 1980.

Accepted July 16, 1980.

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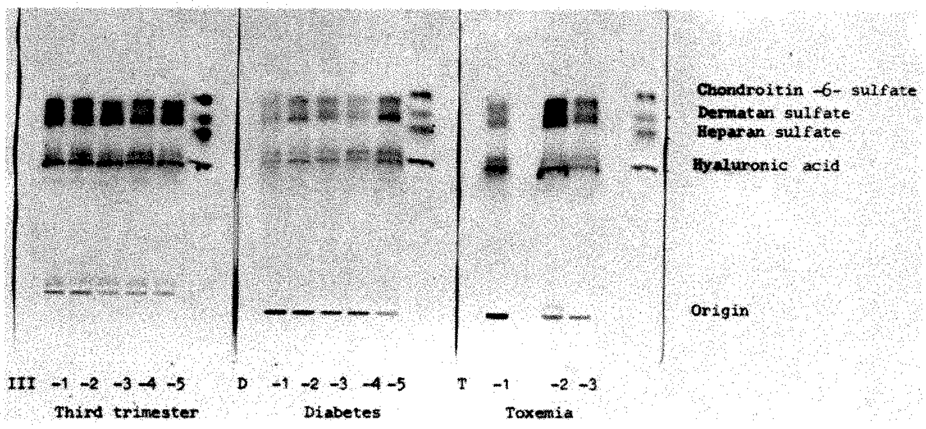

Fig. 1. Electrophoretic separation of GAG in crude preparations from normal, diabetic, and toxemic term placentas. The separation was carried out on cellulose acetate in 0.1M cadmium acetate buffer, pH 4.0, 0.8 mA/cm, 1 to 2 hours. The figures at the bottom represent the number of preparations under each condition (see Table I).

Table I. Yield and composition of crude GAG preparations from normal, diabetic, and toxemic term placentas

Kind of placenta	*No. of placentas*	*Yield of crude GAG preparation in dry defatted tissue (%)*	*Uronic acid in GAG preparation (%)*	*Protein in GAG preparation (%)*	*Uronic acid in dry defatted tissue (%)*
Normal (Third trimester)	1	1.74	9.6	20.0	0.17
	2	1.93	8.3	24.0	0.16
	3	1.68	12.5	15.0	0.21
	4	1.74	10.4	20.0	0.18
	5	1.38	10.8	17.0	0.15
Mean ± SD		1.69 ± 0.20	10.3 ± 1.6	19.2 ± 3.4	0.17 ± 0.02
Diabetic	1	2.95	7.5	9.0	0.22
	2	2.43	16.7	6.2	0.41
	3	2.66	9.2	2.7	0.24
	4	2.13	16.7	*	0.36
	5	1.95	12.5	9.0	0.24
Mean ± SD		2.42 ± 0.40	12.5 ± 4.2	6.7 ± 4.8	0.29 ± 0.08
Toxemic	1	2.75	7.1	4.1	0.19
	2	1.92	13.3	2.1	0.25
	3	1.61	10.4	5.5	0.17
Mean ± SD		2.09 ± 0.59	10.3 ± 3.1	3.9 ± 1.7	0.20 ± 0.04

*Not determined.

natant with three volumes of cold 5% potassium acetate in absolute ethanol. The precipitate was then treated with deoxyribonuclease (Schwarz/Mann) and ribonuclease (Calbiochem), lyophilized, and redissolved in water, and the GAG were precipitated as described above. Uronic acid was estimated by the Bitter and Muir[5] method and protein was determined according to Lowry and associates,[6] with bovine serum albumin (Nutritional Biochemicals) used as the standard.

Electrophoretic separation of GAG was performed on cellulose acetate strips in 0.1M cadmium acetate buffer, pH 4.0, at 0.8 mA/cm for 1 to 2 hours.[1, 4] The electrophoretograms were stained with 0.2% alcian blue 8 GN (Matheson, Coleman & Bell) in ethanol: glacial acetic acid: water (100:50:850) and cleared in 7.5% acetic acid. The proportion of individual GAG on each strip was determined by densitometric scanning at 525 nm with Helena autoscanner. Pure GAG standards were provided by Dr. Cifonelli, University of Chicago.

Electrophoretic separation of GAG preparations also was performed after enzymatic treatment either with testicular hyaluronidase (Sigma Chemical Co.) or with chondroitinase ABC (Seikagaku Kogyo Co.) as described in another work.[1] GAG fractions were obtained by extraction with water from other electrophoretograms, after staining with 0.05% toluidine blue in 80% ethanol. These eluates were hydrolyzed in 4N HCl at 100° C for 6 hours and served for the identification

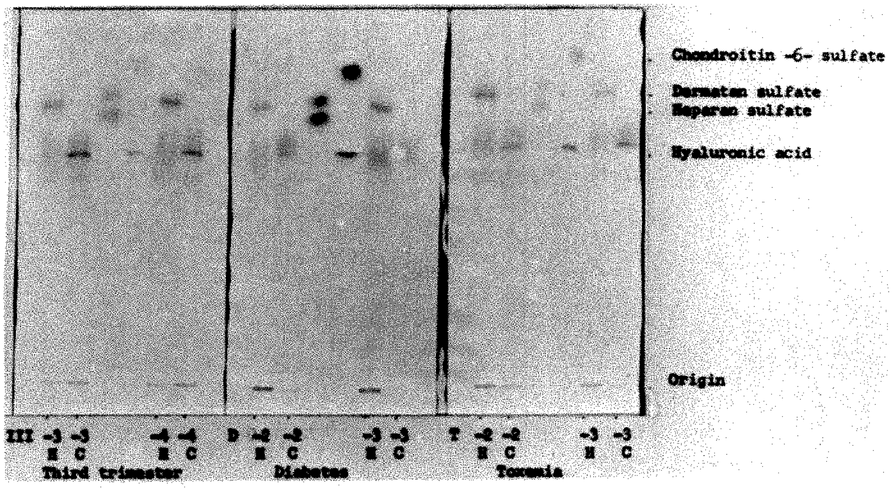

Fig. 2. Electrophoretic separation of GAG in preparations after digestion with testicular hyaluronidase *(H)* and chondroitinase ABC *(C)*. Conditions and signs are the same as those described in Fig. 1.

Table II. Percentages of individual GAG in normal and pathologic term placentas

Kind of placenta	*GAG*	*Percentage (Mean ± SD)*	*Comparison to normal placenta (P)*
Normal	Hyaluronic acid	20.3 ± 1.2	—
	Heparan sulfate	15.1 ± 1.0	—
	Dermatan sulfate	34.2 ± 2.5	—
	Chondroitin 4/6 sulfate	30.3 ± 2.5	—
Diabetic	Hyaluronic acid	23.3 ± 2.9	0.02
	Heparan sulfate	18.0 ± 1.4	0.005
	Dermatan sulfate	30.7 ± 1.1	0.05
	Chondroitin 4/6 sulfate	28.0 ± 1.9	0.1
Toxemic	Hyaluronic acid	25.5 ± 1.2	0.001
	Heparan sulfate	13.7 ± 3.2	0.3
	Dermatan sulfate	28.7 ± 1.9	0.02
	Chondroitin 4/6 sulfate	32.2 ± 3.3	0.3

of hexosamines by paper chromatography, as described in another work.[1].

Results

Uronic acid and protein percentages in crude preparations and their yield by weight from dry defatted placental powder, as well as total uronic acid percentages in dry defatted tissue, are listed in Table I. Yields of crude preparations were found to be higher in diabetic placentas and only slightly elevated in the toxemic ones, as compared to normal tissues. The uronic acid concentration in the preparation did not appear to differ, whereas the protein percentage was markedly lower in the abnormal placentas. The total recovery of uronic acid from dry defatted tissue was higher in diabetic placentas ($p = 0.05$) and not significantly increased in the toxemic ones ($p = 0.50$), as compared to normal placentas.

Electrophoretic separation of GAG preparations is shown in Fig. 1. All preparations revealed four bands, two of which (bands 1 and 4) corresponded to hyaluronic acid (HA) and chondroitin 4/6 sulfate (C4/6S), respectively. Band 2 was positioned close to HA and band 3 appeared between heparan sulfate (HS) and dermatan sulfate (DS). The identity of these two bands was further examined by electrophoresis of the unsusceptible GAG after treatment with either testicular hyaluronidase or chondroitinase ABC. These electrophoretograms are presented in Fig. 2. Bands 2 and 3 remained at their original positions after treatment with testicular hyaluronidase, possibly indicating the presence of HS and DS, respectively, while bands 1 (HA) and

4 (C4/6S) disappeared. Degradation with chondroitinase ABC resulted in the complete disappearance of band 3 (apparently DS) and band 4 (C4/6S). The remaining bands 2 and 1 presumably represent HS and HA. The latter has been shown to be partially resistant to chondroitinase ABC.[1]

Additional evidence for the identity of HS and DS was provided by hexosamine analysis of eluates from electrophoretograms after treatment with testicular hyaluronidase, by means of paper chromatography. One spot corresponding to galactosamine was obtained from band 3, thus confirming its identity as DS. Glucosamine was the major spot recovered from band 2, suggesting the presence of HS. The weaker spot corresponding to galactosamine (band 2) presumably could result from partially degraded DS.[2]

The mean proportions of the four individual GAG, namely, HA, HS, DS, and C4/6S, obtained by scanning densitometry of electrophoretograms carried out in triplicate from diabetic, toxemic, and normal placentas are presented in Table II. It can be seen that the percentage of HA was higher in diabetic and toxemic placentas as compared to normal ones and that HS was higher in diabetic placentas and not significantly different in the toxemic placentas. The proportion of DS was lower in both abnormal placentas while that of C4/6S did not appear to change markedly.

Comment

Although diabetes and toxemia are common complications of pregnancy, their histopathologic manifestations are not specific and also can be found in other abnormalities. In fact, the histopathologic changes observed in human placentas from diabetic and toxemic pregnancies have occasionally been found to a lesser degree in normal placentas at term, indicating that the changes in pathologic tissues differ from the normal quantitatively rather than qualitatively.[7, 8] The alterations reported in the morphology of toxemic[7, 9] and diabetic[8] placentas are very similar and consist of the following: disturbances in villous maturation, focal syncytiotrophoblastic necrosis, marked proliferation of cytotrophoblast, thickening of the villous basement membrane, deposition of fibrin, edema of the villi, and occasional thrombus formation in villi and in the intervillous spaces. The proliferation of cytotrophoblastic cells in the two conditions could be regarded as a reaction of the tissue to hypoxia resulting from the reduced maternal placental blood supply[7, 9] in toxemia and to other factors in diabetes.[8, 10]

Some correlation can be found between the histopathologic changes reported for diabetic and toxemic placentas and the alterations in total GAG content and the proportional distribution of individual compounds in abnormal placentas.

A marked increase in total GAG content could be seen in diabetic placentas and a less significant increase occurred in the toxemic placentas. Hypoxia is usually accompanied by an increase in the rate of GAG synthesis, as observed in various tissues,[11] and is believed to be a nonspecific reaction of the mesenchyme to reduced oxygen tension. The changes found by us in GAG content of toxemic placentas, as compared to normal ones, could be a reflection of this reaction. In diabetes these changes were proposed to be a defensive mechanism in which the placenta itself controls the large amounts of glucose by diverting the glucose to the synthesis of polysaccharides including GAG.[12] In this regard it should be noted that an increase in GAG content in the serum and placenta[13] as well as in urine[14] of the toxemic woman has been reported and has been related to the hypoxic conditions associated with toxemia. A rise in the amount of GAG also has been observed in the urine of diabetic women[14] and in the placentas of rats with chemically induced diabetes.[12] The marked increase in the proportions of HA in diabetic and toxemic placentas (Table II) may be related to the edema observed frequently in the villi.[8] Retention of water in the tissues, which is a common symptom in toxemia, has been correlated with the total increase in GAG concentration[14] and may be connected to the role of HA in swelling processes.[15]

The amount of DS was considerably reduced in diabetic and toxemic placentas. No change in the percentage of HS was observed in toxemia, while the diabetic placenta showed a slight increase. DS and HS have anticoagulant activity and a decrease in their amount could enhance local blood coagulation. It should be noted in this regard that normal pregnancy is accompanied by alterations in the blood clotting mechanisms, leading to a hypercoagulable state.[16] In toxemia these conditions are known to be aggravated and the risk of blood clotting is greater, especially in the placental and renal circulations.[16, 17] Moreover, thrombi and depositions of fibrin have been found in histologic preparations from toxemic[7] and diabetic[8] placentas.

The elevated GAG content and the low percentage of protein in toxemic and diabetic placentas resemble the pattern reported for young placentas.[1] This similarity can be related to the disturbances in placental maturation reported to occur in these conditions, as deduced from the abundance of cytotrophoblastic cells characterizing placentas of early gestational ages.[8, 9]

Although some correlations have been shown between the changes in the GAG patterns and the histopathologic manifestations in toxemic and diabetic placentas, it is not clear whether the alterations in these compounds are of etiologic significance or are a mere consequence of the impaired biological conditions.

We wish to thank Mrs. Y. Herzig for performing the densitometric scanning.

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Maternal alcohol consumption, birth weight, and minor physical anomalies

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The effect of maternal alcohol consumption during pregnancy upon neonatal outcome was examined in 278 mother-infant dyads. Accounts of the mother's drug use as well as information about confounding factors were obtained by personal interviews on two occasions during pregnancy and again after delivery. The infants were examined by a standardized procedure for the presence of minor physical anomalies. Alcohol, in moderate amounts, was found to be unrelated to infant birth weight, birth length, or head circumference. Minor physical anomalies associated with fetal alcohol syndrome were no more common in infants exposed to heavy amounts of alcohol in the first trimester than in nonexposed infants. A weak but significant negative correlation between alcohol consumption and gestational age was not accounted for by covariance with emotional stress, parity, socioeconomic status, mother's age, or use of other drugs. (AM. J. OBSTET. GYNECOL. 138:774, 1980.)

DURING THE PAST DECADE a causal relationship between alcohol abuse during pregnancy and the fetal alcohol syndrome (FAS) has been well documented in the medical literature. The association between growth retardation and a maternal history of alcohol consumption was observed clinically in infants in the first year of life.[1] The characteristic features of the syndrome, with growth deficiency and mental retardation as primary factors, became established through examination of older children and the retrospective pregnancy histories of their mothers.[2, 3] In a recent review Clarren and Smith[4] estimated the magnitude of the problem to be 1 to 2 infants with FAS per 1,000 births.

Although the adverse effects of heavy alcohol exposure on neonatal development are generally accepted, the effects of prenatal exposure to moderate amounts of alcohol have remained unclear. In a large survey of 9,236 women in France, Kaminski and associates[5] found an increase in stillbirths, lowered birth weights, more small–for–gestational age infants (after nicotine was taken into account), and decreased placental weights in infants of mothers who drank more than an average of 1.6 ounces of absolute alcohol (AA) per day. He found no difference between infants born to women who drank up to 1.6 ounces of AA per day and infants of abstainers. In a prospective study of women from core-city Boston, Ouellette and associates[6] reported a significantly higher percentage of infants born prematurely, postmaturely, small for gestational age, with increased major and minor anomalies, and with decreased birth weight, length, and head circumference to women who were heavy drinkers (more than 1.5 ounces of AA per day and at least five drinks on occasion) but no difference between infants of moderate and rare drinkers. Infants of women who decreased alcohol intake during pregnancy were less affected, thus implicating exposure throughout pregnancy or in the last trimester as the vulnerable period. Confounding variables were not accounted for in this study. In contrast, other investigators have suggested that moderate amounts of alcohol use may have adverse effects. Little[7] found a significant linear relationship between the amounts of alcohol consumed and decreased birth

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Supported in part by funds from the National Council on Alcoholism, the University of Colorado Alcohol Research Center, the Developmental Psychobiology Research Group of the Department of Psychiatry, and the Office of Student Aid, University of Colorado Medical School.

Received for publication March 4, 1980.

Revised June 2, 1980.

Accepted July 22, 1980.

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weights when nicotine, parity, gestational age, sex of child, and mother's height were taken into account. Hanson and associates[8] also reported an increase in FAS-like features in infants of women who reported intake of 1 to 2 ounces of AA per day in the month preceding recognition of pregnancy as compared with light-drinking or abstaining control subjects. Unlike the Boston study, which implicated the last trimester as the vulnerable period, Little's and Hanson's studies suggested that exposure during the very early period of morphogenesis was critical. The authors interpreted their findings as suggesting a linear dose-response curve.

A safe level of alcohol consumption during pregnancy has not been established nor has the possibility of a differential effect upon the fetus at various developmental stages been examined. Criteria for precise descriptions of the characteristics of the FAS, particularly for diagnosis in the newborn infant, have not been established.

The purpose of our investigation was to examine the effects of socially prevalent amounts of alcohol consumption upon the partial expression of the FAS. The questions addressed were: Is there a dose-related relationship between the mother's alcohol consumption and retardation of fetal growth when confounding variables have been taken into account? Is there a threshold effect? Is the fetus more vulnerable to weight loss or developmental anomalies if exposure to alcohol occurs at particular stages of gestation? Is there a dose-related increase in minor physical anomalies?

Methods

Population. We interviewed 604 women before 20 weeks of pregnancy. The population was drawn from two public hospital outpatient obstetric clinics. Of these, 337 were observed through delivery. Attrition from causes such as dropping out of the medical care program or delivery at other hospitals, moving, or change of name, occurred in 139 cases; delivery had not occurred in 128 cases at the time the study was terminated. The number of infant-mother dyads in the final data analysis was 278.

The demographics of the population are given in Table I. The subjects were drawn from the core city and from the industrial suburbs. They were predominantly of middle and lower middle class, as estimated by a modification of the Hollingshead scale,[9] with occupation of head of household and mother's educational level used as indicators. The mean age of the group (22 years) was lower than the national average for maternal age (24 years). Thirty-eight percent of the women were between 14 and 21 years of age.

Table I. Demographics of 278 subjects drawn from obstetric clinic populations in two public hospitals

Demographic variable	%
Social class:	
Upper	0.4
Upper middle	2.5
Middle	53.2
Lower middle	37.4
Lower	5.8
Ethnic group:	
Caucasian	69.1
Hispanic	14.0
Black	11.9
Am. Indian	2.2
Oriental	2.9
Age (yr):	
≤ 17	12.9
18-20	23.7
21-30	51.8
31-34	8.6
≥ 35	2.9
Gravidity:	
1	32.7
2	29.1
3	18.3
4	8.6
≥ 5	10.8

Maternal histories. The women were interviewed on three occasions: before 20 weeks' gestation, at 6 to 7 months, and after delivery. The patients signed an informed consent which stated that the study was an investigation of the effects of consumption of moderate amounts of alcohol upon fetal development. By means of a structured, open-ended questionnaire, we asked about use of caffeine, nicotine, prescribed and over-the-counter drugs, illnesses, fevers, exposure to radiation and environmental hazards suspected as teratogens, emotional and social problems, dietary intake, alcohol, and illicit drugs. Questions regarding alcohol use were asked last, after the patient had an opportunity to discuss all other aspects of the pregnancy. The woman was asked to report the last time she consumed alcohol and the kind and number of drinks. Progressing in reverse from the most recent occasion through the preceding months, an attempt was made to record each drinking episode or to establish the regular pattern of alcohol use. In addition, the women were specifically queried about exceptional occasions of heavy consumption such as holidays or celebrations. Additional information about prepregnancy medical history, weight gain, and medical course of this pregnancy and delivery was obtained from the medical charts.

Infant examinations. Prior to the onset of the alcohol study, we had developed a comprehensive stan-

Table II. Distribution of alcohol consumption of 278 pregnant women for the entire pregnancy and for the first and third trimesters

Time during pregnancy	*Total alcohol consumption by interval (ounces of AA)*	*Subjects*		*Mean (Ounces of AA)*	*SD*
		No.	*%*		
Total pregnancy:					
Abstain	0	74	27	0	0
Rare	1-27	144	52	7	6
Moderate-light	28-130	51	18	63	28
Moderate-heavy	131-240	4	02	188	24
Heavy	>240	5	02	856	426
First trimester:					
Abstain	0	107	39	0	0
Rare	1-9	104	37	3	2
Moderate-light	10-43	43	15	22	9
Moderate heavy	44-80	15	05	58	7
Heavy	>80	9	03	408	365
Third trimester:					
Abstain	0	148	53	0	0
Rare	1-9	91	32	3	2
Moderate-light	10-43	31	11	22	9
Moderate-heavy	44-80	4	01	60	11
Heavy	>80	4	01	120	38

Table III. Relationship between first- and third-trimester use of drugs

Drug	*First trimester*		*Third trimester*		*T value*
	Mean (12 wk)	*SD*	*Mean (12 wk)*	*SD*	
Alcohol (ounces)	21	95	6	18	2.66*
Nicotine (ml)	1,826	1,827	741	1,158	8.46*
Caffeine (mg)	46	56	22	32	9.80*
Marihuana (cigarettes)	34	119	19	81	4.48*

*$p < 0.01$.

dardized examination for minor physical anomalies detectable by surface examination. The pilot sample consisted of 200 infants randomly selected from the hospital nursery. The list of 93 anomalies was compiled from the literature on minor physical anomalies[10, 11] and from descriptions of the FAS. In addition to standard measurements of head and length, we measured upper lip and palpebral fissure length, inner canthal distances, diameter of hemangioma and fontanelles, angle of ear slant, angle of clinodactyly, and extension of extremities.

Information regarding birth weight, clinical gestational age, Apgar scores, prenatal medical complications, and major anomalies was obtained from the medical record. The infant's gestational age was calculated from the mother's report of last menstrual period. If the calculated gestational age was more than 3 weeks different from the infant's clinically estimated gestational age, the clinical estimate was used.

All infants were more than 24 hours of age at the time of the examination. The examiner was unaware of the mother's pregnancy history with one exception when a nurse inadvertently revealed that the mother was a heavy drinker.

Results

We computed a score for the quantity of each drug used. For alcohol, we multiplied the amount of absolute alcohol in each kind of beverage (beer, wine, or distilled) by the number of drinks per occasion. For example, one mixed drink of 90 proof liquor was calculated as 0.67 ounce of AA. An occasion was defined as the day of gestation on which the drinking occurred. We then summed the amounts of alcohol for all occasions within an interval such as a trimester or for the entire pregnancy. The same method of relating the time of fetal exposure to the day of gestation was used for all the drugs. The caffeine score was the amount of caffeine in each kind of beverage (coffee, tea, or cola) times the number of cups per day. The nicotine score was the number of milligrams per cigarette in the brand smoked times the number of cigarettes per day. The marihuana score was the number of cigarettes per occasion. Other illicit drugs were too rare to be treated quantitatively.

As is shown in Table II, approximately 80% of the population abstained or rarely used alcohol. Two percent of the population averaged more than 1½ drinks per day for the entire pregnancy; 3% were in this cate-

Table IV. Stepwise multiple regression of birth weight and birth length on nine independent variables for 278 mother-infant dyads

Birth weight		*Birth length*	
Variable	*% of variance explained*	*Variable*	*% of variance explained*
Gestational age	23*	Gestational age	22*
Mother's prepregnancy weight	29†	Total nicotine	28*
No. of minor physical anomalies	33†	Parity	29‡
Total nicotine exposure	36†	Mother's height	29
Parity	37†	Total caffeine	30
Total marihuana	38	Total alcohol	31
Total caffeine	38	Socioeconomic status	31
Mother's age	38	Mother's age	31
Socioeconomic status	38	Total marihuana	31
Total alcohol	38		

*$p < 0.001$.
†$p < 0.01$.
‡$p < 0.05$.

gory in the first trimester. Twenty percent were considered to be moderate drinkers whose alcohol consumption averaged from one drink per week to 1½ drink per day. This method of averaging obscures the blood levels of alcohol to which an infant might actually have been exposed. Of the five women who were categorized as heavy drinkers in the first trimester, four reported peak consumption of 11 to 40 drinks during an 8-hour period. Of the 24 women categorized as moderate to heavy drinkers, the majority had patterns of intermittent drinking on social occasions in amounts sufficient to average the given levels.

Our method of selecting the study population by recruiting women who sought prenatal care in the first trimester may have biased against the inclusion of heavy alcoholics. Since our objective was to assess the effects of moderate and socially prevalent levels of alcohol, the study design was intended to maximize data about alcohol use rather than about cases of high consumption.

A significant reduction in usage of all drugs occurred in the third trimester as compared with the first (Table III).

Alcohol consumption was significantly correlated with nicotine ($r = 0.30$) and caffeine ($r = 0.15$) but not with marihuana ($r = 0.04$).

Birth weight. The effect of alcohol upon infant birth weight was analyzed by a stepwise multiple regression equation. First, we included in the equation four variables known to affect birth weight—gestational age, mother's prepregnancy weight, parity, and nicotine—and the three drugs—alcohol, caffeine, and marihuana. The known variables contributed significantly to the variance in birth weight but alcohol did not. For exploratory purposes we recalculated the equation with additional variables we had included in our data base. These were mother's age, socioeconomic status, weight gain during pregnancy, and diet and the number of anomalies in the infant. Alcohol did not contribute significantly to the variance in birth weight in any of the calculations. The correlations of alcohol with weight gain and with diet were no different from zero. Therefore, these two variables had no effect upon the association between birth weight and alcohol.

Table V. Stepwise multiple regression of gestational age on nine independent variables for 278 mother-infant dyads

Gestational age	
Variable	*% of variance explained*
Total alcohol	02*
Mother's age	03
No. of anomalies	04
Total nicotine	05
Total marihuana	05
Socioeconomic status	05
Stress	05
Parity	05
Total caffeine	05

*$p = 0.01$.

The order in which variables were accepted into a final equation is given in Table IV. The first five variables—gestational age, mother's prepregnancy weight, number of minor physical anomalies, total nicotine, and parity—explained approximately 37% of the variance in birth weight. Alcohol was the last variable accepted into the equation.

Because alcohol consumption was greatest during

Table VI. Relationship of characteristics associated with FAS to maternal alcohol consumption during pregnancy

	Alcohol group				
FAS anomaly	*1. Abstain (74 women)*	*2. Rare (144 women)*	*3. Moderate to light (51 women)*	*4. Moderate to heavy (4 women)*	*5. Heavy (5 women)*
Short palpebral fissure	3	0	1	0	0
Maxillary hypoplasia	1	0	0	0	0
Upturned nose	6	12	6	1	0
Hypoplastic philtrum	6	8	4	0	0
Wide inner canthal distance	3	1	0	0	0
Long upper lip	11	3	0	1	0
Jitteriness	14	30	9	0	1

the first trimester and because weight gain is relatively greatest in the third trimester, we used the same method to analyze the contribution of the first and the third-trimester drug consumption separately upon birth weight. The results for alcohol were no different from the statistics derived from the total pregnancy scores.

Other growth measures. A similar analysis was used to examine the effect of drugs upon birth length with mother's height substituted for mother's prepregnancy weight. Twenty-eight percent of the variance was explained by three of the independent variables—gestational age, total nicotine and parity (Table IV). None of the other independent variables contributed significantly to the prediction of birth length.

Because infants with FAS are characteristically small for gestational age we also performed a multiple regression equation on percentile weights. Again alcohol did not contribute significantly to the variance.

Finally, we examined the effect of the variables that had been entered in the birth weight equation upon head circumference. There was no significant alcohol effect.

Gestational age. A stepwise multiple regression analysis also was used to examine the effects of the drugs upon gestational age. The variables entered in the equation in addition to total alcohol were: parity, age, socioeconomic status, number of infant anomalies, total nicotine, total caffeine, and total marihuana. Alcohol, the only variable contributing significantly to the variance in gestational age, was accepted first by the equation, explaining only 2% of the variance. The correlation between alcohol and gestational age was −0.14 suggesting a weak relationship.

Emotional stress also has been implicated in premature deliveries[12] and may be a factor in alcohol consumption. In our study, women who had sought or been referred to a psychiatrist or social worker for problems during pregnancy or who had experienced an extraordinary trauma, such as death in the immediate family, had been coded as emotionally stressed for comparison with nonstressed women. We recomputed the multiple regression on gestational age, and added this measure of emotional stress as an independent variable. Stress was found to be positively correlated with total alcohol consumption ($r = 0.24$), but it did not contribute significantly to the variance in gestational age. The order of acceptance of nine variables entered in the equation is given in Table V.

Minor physical anomalies. Before testing the relationship between alcohol consumption and minor anomalies, we examined the effects of gestational age and race upon each anomaly.

Four anomalies were excluded on the basis of race. Epicanthal folds and slanted eyes were found to be disproportionately high in Oriental and Hispanic infants; slanted ears were disproportionately high in blacks, and clinodactyly was higher in Caucasians. Epicanthal folds and slanted ears have been reported in 26% to 50% of infants with FAS, clinodactyly and slanted eyes have been reported only occasionally.

Our analysis by gestational age indicated that one anomaly, hypoplastic labia, occurred with higher frequency in infants under 38 weeks' gestation. The measured anomalies, i.e., palpebral fissure, inner canthal distance, and length of upper lip, were standardized for gestational age. Measurements outside two standard deviations were considered abnormal.

The mean number of anomalies after correction for race and gestational age was 3.4 (SD = 2.1).

After making the above corrections, we entered the total number of anomalies in a stepwise multiple regression equation with nicotine, caffeine, marihuana, and alcohol as the independent variables. None of the variance in total number of minor physical anomalies was accounted for by the drugs, when either total consumption or first-trimester use was considered. We found that the correlation between the total number of anomalies and the alcohol consumption in the first trimester, when anomalies were expected to develop and drug consumption was highest, was no different from zero (r = −0.06).

It was possible that the correlation analysis might have masked the effects of alcohol by too broad an inclusion of minor anomalies unrelated to alcohol or by the undue influence of a few outlying cases. Therefore, we used the five levels of alcohol consumption given in Table II to examine the frequency of seven items selected as typically associated with the growth retardation of FAS. The frequencies of these characteristics—short palpebral fissures, maxillary hypoplasia, upturned nose, hypoplastic philtrum, wide inner canthal distance, long upper lip, and jitteriness—are given for the five classifications of alcohol in Table VI. Chi-square analysis indicated that there were no significant differences among levels of alcohol consumption for any of the anomalies.

Comment

The results of this study do not support a linear dose-response relationship between moderate alcohol consumption and decreased birth weight. The difference in our findings from previous reports in the literature may be a function of population differences, of sample size, or of research design. Apparently, our population did not differ substantially from previous studies in the proportion of women classified as moderate to heavy alcohol consumers in early pregnancy. Five percent of our population as compared with 5% to 9% in other studies[6–8] consumed an average of at least 0.67 ounce of AA per day in the first trimester.

Although the total number of women we interviewed was relatively small, in a sample size of 278 cases, statistical relationships are expected to be fairly stable. Twenty-one percent of the population (N = 60) who were classified as moderate to heavy drinkers represented a sufficiently large number for statistical comparison of differences between moderate and abstaining or rare users.

The results also indicated that the population size was adequate to measure the effects on birth weight of the confounding variables—gestational age, parity, and nicotine. Thirty-one percent of the women (N = 84) smoked an average of half a pack or more of cigarettes per day; 13% of these were heavy users of alcohol. Since alcohol and nicotine were significantly correlated, the effect upon birth weight appeared to be redundant. This finding is at variance with effects of alcohol reported by Little[7] and Kaminski and associates[5] after nicotine had been accounted for by separating smokers and nonsmokers. The results of our stepwise multiple regression suggested that after confounding variables were entered in the equation the effect of alcohol consumption was negligible.

Another consideration that may account for the difference in our findings from other studies of moderate drinkers is the marked decrease in alcohol consumption in the third trimester. Although we did not include a systematic inquiry into the reasons consumption was decreased, it appeared to be due to information about adverse effects obtained from the media and from medical personnel and an increased awareness resulting from our research.

The finding that decreased gestational age is related to increased levels of moderate alcohol consumption needs further investigation. We were unable to explain the relationship by implicating emotional stress. A number of other factors, known to contribute to preterm deliveries, were not included in our analysis. Maternal age less than 14 years, frequent close-spaced pregnancies, the black race, various obstetric complications, including maternal infections, and gestational age of previous infants born to the same women are among the factors that we have not examined.[13] As in the case of birth weight, adequate information about confounding factors might alter the suggested impact of alcohol as causally related to gestational age at delivery. Nevertheless, this finding has been reported by other investigators.[5, 14] Further investigations may contribute to explaining the presumptive evidence of a relationship between moderate alcohol use and small infants.

The unique contribution of this study was the standardization of the infant examination for minor physical anomalies as a partial expression of FAS features. The validity of the examination and its potential usefulness are suggested by the finding that the number of minor physical anomalies explains 5% of the variance in birth weight. The measure may identify infants who were subject to intrauterine disturbances in growth and development. If a common factor influenced both birth weight and the number of minor physical anomalies, the results of the regression equations suggested that the common factor was not maternal ingestion of alcohol. A further analysis of other pregnancy and perinatal factors related to anomalies will be reported in a separate communication.

No new or additional information about very heavy alcohol consumption or about the FAS can be derived from this investigation. The lack of a partial expression of the syndrome in moderate drinkers suggests that a log of the dose response or a threshold effect might be hypothesized as more probable than a linear relationship.

In summary, moderate amounts of maternal alcohol consumption were found to have no effect upon birth weight and not to be related to an increased incidence of minor physical anomalies. An indication that moderate alcohol consumption is related significantly to premature delivery requires further investigation.

We thank Drs. Lula Lubchenco and Linda Morgan for pediatric consultations, Dr. Donald Stilson for statistical consultations, Caroline Augustyn and Jane Streigle for research assistance, Nanci Avitable for computer programming, and the nurses and staff of the obstetric clinics and nurseries in Colorado General Hospital and Denver General Hospital for their cooperation.

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The Northwestern University multihospital twin study

I. A description of 588 twin pregnancies and associated pregnancy loss, 1971 to 1975

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Five hundred eighty-eight women who were delivered of twins at 13 hospitals affiliated with the Northwestern University Medical School or its outreach institutions between 1971 and 1975 were studied. Eighty-six of the infants did not survive the pregnancy, delivery, or the first week post partum. Maternal demographic and obstetric characteristics are outlined. Infants are categorized by birth order and by birth weight. The mean weight (and standard deviation) of first twins was 2,388 gm (±742 gm) and that of second twins was 2,314 gm (± 766 gm). The rate of pregnancy wastage was highest in mothers who were under 20 years of age, who were of low parity, and who were delivered prior to the physiologic maturation of the fetus. The crude mortality rate of twin 1 was 6.1/100 versus 8.5/100 for twin 2. The causes of death in all infants over 2,000 gm are listed. All pregnancy losses are categorized into unavoidable deaths (26.6%), probably not avoidable deaths (59.4%), and possibly avoidable deaths (13.9%). (AM. J. OBSTET GYNECOL. 138:781, 1980.)

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Supported in part by The Charles A. Fields Medical Foundation, Ltd., The Center for Study of Multiple Gestation, The Center for the Advancement of Reproductive Health, Northwestern University Computer Center, and Medical Research Consultants, Inc.

Presented at a meeting of The Chicago Gynecological Society, Chicago, Illinois.

Received for publication September 17, 1979.

Revised July 9, 1980.

Accepted July 21, 1980.

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RECENT ADVANCES in obstetric practice and newborn care have dramatically improved perinatal survival among various types of high-risk pregnancy. Nonetheless, the risk of pregnancy loss in twin gestation remains three or more times that of singleton pregnancy.[1-3] This risk includes death in one or both infants by spontaneous abortion or antepartum death as well as intrapartum and postpartum deaths. The respiratory distress syndrome (RDS) is the most common cause of death.[4, 5] Even though twin gestations represent only about 1% of all gestations on the average, they contribute disproportionately to the total perinatal morbidity and mortality in the United States and elsewhere.[5-8]

In November, 1975, a state-supported regional perinatal unit began providing services at the Prentice Women's Hospital and Maternity Center in Chicago, Illinois. This hospital, a member of the McGaw Medical

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Table I. Participating hospitals and number and percentage of mothers in the study

	Mothers delivered of twins	
	No.	*%*
Wesley-Passavant/Prentice	125	21.3
Evanston	86	14.6
Other hospitals (Code No.):		
1	82	13.9
2	49	8.3
3	44	7.5
4	43	7.3
5	39	6.6
6	32	5.4
7	32	5.4
8	29	4.9
9	13	2.2
10	9	1.5
11	5	0.9
Total	588	100.0

Complex of Northwestern University, provides level III perinatal services to a network of 24 outlying hospitals. The amalgamation of the obstetric services at Wesley Memorial Hospital, Passavant Memorial Hospital, and the Chicago Maternity Center formed the Prentice Women's Hospital and Maternity Center. This analysis of twin gestations and their associated pregnancy loss was undertaken for the 5-year period preceding the formal opening of the perinatal unit. These data form a baseline against which future data could be compared.

Material and methods

Participating hospitals. Two Northwestern University Hospitals—the Wesley-Passavant/Prentice Hospital complex and Evanston Hospital—plus 11 other hospitals agreed to participate in the study. Of this group, six have or had a formal affiliation with Northwestern University. All hospitals are located in central Chicago or its northern and western suburbs. Patients attending each institution come from mixed socioeconomic classes. Table I lists each of the participating hospitals by a code number as well as the numbers of mothers delivered of twins during the study period.

Data collection and processing. A data collection instrument was prepared with certain antenatal, intrapartum, and postpartum factors known or suspected to contribute to pregnancy wastage taken into consideration. Categories of information included medical as well as socioeconomic and demographic data. A physician-directed team of five obstetric nurses was trained to review charts and record data on a standardized form. The team made efforts to locate and review records of all twin deliveries at these cooperating institutions. Successful transcription of all twin records occurred in 12 of the 13 hospitals.

Table II. Maternal demographic characteristics

Characteristic	*No.*	*%*
Age (yr):		
15-20	66	11.2
21-24	142	24.1
25-30	247	42.0
31-34	77	13.1
35+	53	9.0
Not stated	3	0.5
Race/ethnic group:		
White	372	63.3
Black	159	27.0
Spanish-American	22	3.7
Oriental	4	0.7
Not stated	31	5.3
Marital status:		
Married	449	76.4
Previously married	47	8.0
Never married	81	13.8
Not stated	11	1.9
Religion:		
Protestant	129	21.9
Catholic	219	37.2
Jewish	38	6.5
Other not stated	202	34.4
All women	588	100.0

The completed forms were visually scanned, keypunched, verified, and then checked for possible errors by a computer program. Whenever errors were suspected, the hospital records were reviewed for a second time, either to verify the originally coded data or to correct the error. Data processing and analyses were performed under the auspices of the Vogelbach Computer Center of Northwestern University.

Results

During the 5-year study period, 82,229 live births were recorded at the 13 study hospitals. Five hundred eighty-eight women were delivered of twins for a rate of 7.2/1,000 live births or one woman delivered of twins for every 140 puerperal women. When the deliveries of the hospital with the incomplete record review are excluded from analyses, this figure becomes one twin gestation per 134 women delivered. The characteristics of the 588 mothers and their 1,176 offspring, 86 of whom did not survive the pregnancy, delivery, or first week post partum, form the basis of this report.

Mothers of twins. The demographic characteristics of the 588 mothers of twin pairs are summarized in Table II. Approximately two thirds of the mothers were in their 20s and one fourth were in their 30s or 40s. Almost two thirds of the mothers were white and

Table III. Maternal obstetric characteristics

Characteristic	*No.*	*%*
Parity:		
0	167	28.4
1-2	279	47.4
3-4	92	15.6
5+	44	7.6
Not stated	6	1.0
Weeks from LMP:		
21-24	8	1.4
25-28	21	3.5
29-32	35	6.0
33-36	131	22.3
37+	372	63.3
Not stated	21	3.5
Time of twin diagnosis:		
≤24 weeks	54	9.2
25 weeks to onset of labor	262	44.6
Onset of labor to delivery of first twin	57	9.7
After delivery of first twin	89	15.1
Not stated	126	21.4
All women	588	100.0

Table IV. Infant characteristics by birth order

	Twin 1		*Twin 2*	
Characteristic	*No.*	*%*	*No.*	*%*
Sex:				
Male	284	48.3	271	46.1
Female	302	51.4	310	52.7
Not stated	2	0.3	7	1.2
Weight (gm):				
≤499	6	1.0	11	1.9
500-999	24	4.1	22	3.7
1,000-1,499	32	5.4	23	3.9
1,500-1,999	72	12.2	91	15.5
2,000-2,499	154	26.2	162	27.6
2,500-2,999	180	30.6	169	28.7
3,000-3,499	90	15.3	82	13.9
3,500+	24	4.1	17	2.9
Not stated	6	1.0	11	1.9
All infants	588	100.0	588	100.0

Table V. Risk of pregnancy loss by selected maternal characteristics

		Women with pregnancy loss	
Characteristic	*Total women*	*No.*	*Rate/100*
Age (yr):			
15-20	66	14	21.2
21-24	142	16	11.3
25-30	247	22	8.9
31-34	77	2	2.6
35+	53	5	9.4
Not stated	3	–	–
Parity:			
0	167	22	13.2
1-2	279	30	10.8
3-4	92	4	4.3
5+	44	3	6.8
Not stated	6	–	–
Weeks from last menstrual period:			
21-24	8	8	100.0
25-28	21	14	66.7
29-32	35	9	27.7
33-36	131	12	9.2
37+	372	13	3.5
Not stated	21	3	–
Total	588	59	10.0

one fourth were black. Roughly, three of every four women were married at the time of delivery.

Maternal characteristics of obstetric significance are summarized in Table III. Approximately one quarter of the women were nulliparous and approximately half had one or two prior deliveries. Only approximately two thirds of deliveries occurred at term (37 or more weeks of gestation from last menstrual period). Over 20% of the twins were delivered at 33 to 36 weeks of gestation as calculated from the last menstrual period, and approximately 11% of twins were delivered prior to 33 weeks of gestational age.

The time at which the diagnosis of twin gestation was first recorded is also shown in Table III. In almost half of the cases, the diagnosis of multiple gestation was made in the third trimester; however, in 24.8% of the cases the diagnosis was made only after labor had begun or after the first twin had already been delivered. In 126 cases (21.4%), the time of diagnosis was not stated. If these 126 cases are excluded from consideration, then in the remaining cases where the time of diagnosis was known the diagnosis of twins was not suspected until after labor had begun in almost one third of the cases.

Twin infants. For each member of the twin pair, the sex and birth weights are shown in Table IV. Overall, there were 555 male infants, 612 female infants, and nine instances in which the sex either was not reported or was unidentifiable. The predominance of female over male births was apparent for both the first and second twins, with the percentage of male births being slightly less among second twins. Sex ratios for twin 1 and for twin 2 are not significantly different ($p > 0.05$) and not significantly different ($p > 0.05$) from one. It was not possible to assess zygosity in this study, because the individual practitioners who delivered the infants did not uniformly test the infants by acceptable methods.

The distribution of all birth weights in 500 gm categories is also shown in Table IV for the first and second twins. A birth weight of ≥2,500 gm was recorded for 51.0% of first twins and 47.4% of second twins. The mean birth weight for the group of first

Table VI. Twin-specific mortality by birth weight

Weight (gm)	First twin			Second twin		
		Deaths			Deaths	
	Total infants	No.	Rate/100	Total infants	No.	Rate/100
500	6	5	83.3*	11	11	100.0
500-749	9	9	100.0	9	8	88.0
750-999	15	9	60.0	13	9	69.2
1,000-1,249	12	3	25.0	9	5	55.6
1,250-1,499	20	2	10.0	14	3	21.4
1,500-1,999	72	3	4.2	91	6	6.6
2,000-2,499	154	2	1.3	162	3	1.9
2,500-2,999	180	2	1.1	169	1	0.4
3,000-3,499	90	0	–	82	0	–
3,500+	24	0	–	17	0	–
Not stated	6	1	–	11	4	–
Total	588	36	6.1	588	50	8.5

*In the single case where survival was stated, the hospital record was discrepant. The operative note described delivery of twin stillborn infants; the order sheet stated that one infant was transferred to a high-risk center alive. Follow-up at this hospital was unavailable.

Table VII. Causes of death in infants >2,000 gm

Time of death	Birth order	Sex	Weight (gm)	Cause of death
Antepartum	B	Male	2,440	Abruptio placentae, macerated fetus
Antepartum	A	Male	2,665	Unknown cause, macerated fetus
Antepartum	A	Female	2,041	Unknown cause, macerated fetus
Intrapartum	A	Female	2,211	Interlocking head
Intrapartum	B	Female	2,070	Interlocking head
Intrapartum	B	Male	2,495	Prolapsed umbilical cord
Intrapartum	A	Male	2,977	Velamentous cord, vasa previa
Post partum	B	Female	2,155	RDS

twins (2,388 gm ± 742.3) was slightly higher than that for the second twins (2,314 gm ± 766.4). This difference in mean birth weights was not statistically significant ($p > 0.05$).

Risk factors in pregnancy loss. The relationship between selected demographic and obstetric factors for women who suffered a pregnancy loss (death of one or both twins) compared to all of the mothers of twins is shown in Table V. The pregnancy loss rate (uncorrected for weight or other factors) shown in the right-hand column is calculated as:

$$\frac{\text{Number of women with pregnancy loss}}{\text{Total number of women}} \times 100.$$

This rate can be interpreted as the risk of pregnancy wastage to a mother of twins, i.e., one or both twins not surviving the pregnancy, delivery, or neonatal period. With respect to maternal age, the pregnancy loss rate was highest for the youngest women, aged 15 to 20 years (21.2/100), and declined to a minimum (2.6/100) for women in their early 30s, after which it rose again. A similar pattern was observed with respect to parity. The pregnancy loss rate was highest among nulliparous twin mothers, declined to a minimum among women of parity 3 or 4, and increased with increasing parity thereafter.

The relationship of pregnancy loss to gestational age at delivery is clearly documented in the third panel of Table V. While the estimation of gestational interval is always subject to an unknown degree of error, the data are presented here as they were recorded in the clinical records, calculated as weeks from last menstrual period to delivery. The pregnancy loss rate per 100 women declined from a maximum of 100 at 21 to 24 weeks' gestational age to a minimum of 3.5 for term gestations. The complement of the pregnancy loss rate is the survival rate for one or both twins. Thus, in over 90% of twins delivered at 33 to 36 weeks, one or both twins were discharged alive from the hospital, and in approximately three fourths of twin deliveries at 29 to 32 weeks, at least one of the twins was discharged alive from the hospital.

While the gestational interval is obviously one of the most important factors in determining physiologic maturity and the ability of the newborn infant to survive in the extrauterine environment, there is no way to measure this interval without the errors of recall of the dates of last menstrual period and the variability of cycle lengths. Birth weight, on the other hand, which generally can be correlated with gestational age, can be measured easily and with a small degree of error for individual members of each twin pair. The twin-specific mortality rates by birth weight are shown in Table VI. In order to more clearly study mortality among the earliest potentially viable gestations, 250 gm increments were chosen for weight categories <1,500 gm.

For each category of birth weight the total number of infants delivered (or spontaneously aborted) and the number who died (antepartum, intrapartum, and postpartum deaths) are shown. The mortality rate is calculated from these two numbers. For every category of birth weight less than 2,500 gm, except in the 500 to 749 gm group, the second twin had a higher (but not significantly so) mortality rate than the first twin ($p > 0.10$). These differences are reflected in the greater over-all mortality rate for all second twins (8.5/100) compared to that for first twins (6.1/100). As birth weight increased, the mortality rate declined for both first and second twins. A dramatic decline in the mortality rate of first twins occurred at a weight of 1,000 gm. For the second twin, this trend began at 1,250 gm.

Among infants weighing 2,000 gm or more, there were eight deaths (Table VII). Of these, three infants died in the antepartum period and were macerated at birth. One of these apparently died as a result of abruptio placentae. Four of the deaths occurred intrapartum. Two of these infants had interlocking heads and died during attempts at delivery. The other two intrapartum deaths were due to a prolapsed umbilical cord and a velamentous insertion of the cord with vasa previa. The remaining deaths among this group of eight infants occurred post partum and were due to RDS.

Based on current standards of obstetric and neonatal care, it is possible that at least six of the eight deaths in infants weighing greater than 2,000 gm might have been prevented. Similarly, it is possible that all five deaths related to RDS in infants weighing in excess of 1,500 gm might have been avoided, given optimal circumstances.

Review of the deaths by cause and by birth weight suggests that in 23 cases (26.7%) the deaths were not preventable (Table VIII). These deaths occurred in the antepartum period, often of unknown causes, with subsequent maceration of the fetus, or because of severe anomalies incompatible with life. Regardless of the sophistication of medical care available, it is difficult to envision how these could have been avoided. In 51 cases (59.4%), deaths were probably not avoidable. All of these fetuses weighed less than 1,500 gm and death occurred either in the intrapartum or postpartum period, usually because of immaturity and RDS.

Table VIII. Avoidable pregnancy loss in 588 twin gestations

Avoidable death	%	*Cause of death*
No	26.6	Severe anomalies, 2 Antepartum death with weight <1,500 gm, 21
Probably not	59.4	Unknown causes, intrapartum and post partum, with weights <1,500 gm, 49 Unknown causes, antepartum, with macerated fetuses (2,665 and 2,041 gm), 2
Possibly	13.9	RDS with weight >1,500 gm, 5 Abruptio placentae (1,814 and 2,440 gm), 2 Intrapartum asphyxia with interlocking heads (2,211 and 2,070 gm), 2 Velamentous cord with vasa previa (2,977 gm), 1 Cord prolapse (2,495 gm), 1 Unknown cause, intrapartum (1,616 gm), 1

Whether or not individual deaths could have been prevented, it is reasonable to compare these data with those from another American center providing similar care. The results of 242 consecutive twin gestations delivered at Parkland Memorial Hospital in Dallas, Texas, during a similar time period (January, 1971, to July, 1974) is shown along with the Chicago data in Table IX. With the exception of the neonatal mortality rate for infants weighing between 1,500 and 1,999 gm, the stillborn and neonatal death rates for twins were slightly but consistently lower in the 13 Chicago hospitals, although the differences did not attain statistical significance.

Comment

The experience with outcome of twin pregnancies in 13 Chicago hospitals during the 5-year period 1971 to 1975 was reviewed retrospectively. The overall risk of pregnancy loss in twin gestation was 6.6%. Of the various risk factors studied, clearly the most significant is pregnancy termination prior to the physiologic maturation of the fetus. The analysis of mortality by gestational age or birth weight makes this point obvious. When one considers that almost half of all the infants

Table IX. Perinatal mortality rates (%): 13 Chicago hospitals (1971 to 1975) versus Parkland Memorial Hospital (1971 to 1974)

Birth weight (gm)	*13 Chicago hospitals*			*Parkland Memorial Hospital**		
	Total infants	*Stillbirths*	*Neonatal deaths*	*Total infants*	*Stillbirths*	*Neonatal deaths*
<500	17	70.6	23.5	5	100.0	–
500-999	46	13.0	63.0	23	30.4	65.2
1,000-1,499	55	5.5	18.2	46	8.7	30.4
1,500-1,999	163	2.5	3.1	99	3.0	1.0
2,000-2,499	316	0.9	0.6	145	2.8	0.7
2,500+	562	0.2	0.0	166	0.6	0.6
Unknown	17	–	–	–	–	–
Total	1,176	2.9	4.3	484	5.0	6.6
≥1,000 gm	1,113	1.4	1.6	456	2.6	3.7

*Data from Pritchard, J. A., and MacDonald, P. C.: Williams Obstetrics, ed. 15, New York, 1976, Appleton-Century-Crofts, p. 544.

in this series weighed less than 2,500 gm, the clinical relevance of this finding is obvious. By way of comparison with singleton births, in a recent evaluation of 340 twin births and more than 30,000 singleton births the respective numbers of infants with birth weights less than 2,500 gm were 60% for the twins and less than 12% for the singletons.[9]

Given these findings, it is reasonable to ask if anything can be done to decrease the rate of pregnancy wastage among twin gestation. Since the mechanism of the onset of parturition is not clearly understood, it is not yet possible to definitively outline a course of therapy capable of preventing premature labor. The available methods which have been used to accomplish this range from simple treatments such as bed rest at home or in the hospital to the use of pharmacologic agents such as beta-adrenergic drugs and intravenous alcohol to inhibit uterine activity.[5, 6, 10–13] All of these measures have been observed to prolong gestation in certain instances. In particular, bed rest has been shown to be associated with higher birth weights in twins compared with those of twins whose mothers had not been put to bed.[14–17] The mechanism for the increased fetal weight is not yet known, but several factors may be operative, such as improved uterine blood flow, restriction of cigarette smoking, improved maternal nutrition, and possibly reduced intra-abdominal pressure.[17] Although the concept of bed rest is attractive because of its simplicity, it is not as yet universally accepted as being of value.[18] At present, the routine use of bed rest is more prevalent in Europe than in the United States.[14–17] The major factor inhibiting more widespread use of bed rest in the United States is the prohibitive cost of hospitalization. Realistically speaking, attempts to instruct patients who already have children at home to maintain strict bed rest are probably of greater value to the psyche of the physician than to the patient. A nonhospital facility such as a motel or a hotel, located within a few minutes of the hospital and staffed by nurse-midwives, could probably be of great help in our attempts to improve pregnancy outcome in multiple gestation. Bed rest, however, can be of value only if the diagnosis of multiple gestation is made early in pregnancy.[14] In the series we have reviewed, more than one of four cases of twin gestation was not recognized until labor began. The diagnosis of multiple gestation must be suspected clinically before an ultrasonic or other definitive examination can be ordered. At present, few obstetricians advocate routine ultrasound examinations for all pregnant women during the twelfth to sixteenth week in order to make an early diagnosis of multiple gestation. If ultrasound is performed at this early stage of gestation, accurate diagnosis of multiple gestation is facilitated; also, the fact that more twins apparently exist in the first trimester than survive into the third can be appreciated.[19] This discrepancy, following resorption of blighted ova, has led to the concept of the "vanishing twin."[20]

Whenever preterm delivery is threatened or imminent, efforts may be undertaken to (1) inhibit labor with beta-adrenergic stimulating drugs or ethanol[12] and (2) to accelerate fetal pulmonary maturation with exogenous glucocorticoid administration to the mother.[21] This latter therapy has the greatest potential impact in those situations in which the risk of RDS is highest, i.e., between 28 and 32 weeks of gestation. Since it takes approximately 24 to 48 hours until the protective effect of maternal glucocorticoid administration can be observed in the fetus, the use of betamethasone can be advocated only in situations where it is likely that delivery can be delayed at least 24 hours and where its use is not medically contraindicated.

Glucocorticoid therapy is still the subject of intense investigation, since its long-term effects are unknown at this time.

In order to provide the highest standards of intrapartum and neonatal care to the mother of twins and her offspring, referral of these high-risk patients to level III hospitals prior to labor and delivery is clearly indicated. Since in many cases the diagnosis of twin pregnancy is not made until after labor has ensued or until the first twin has been delivered, early transfer of low–birth weight newborn infants to a neonatal intensive care center is recommended to avoid unnecessary death from RDS among those premature infants born in outlying institutions.

The retrospective nature of the study and the number of hospitals involved precluded any attempt to determine the quality of antenatal care provided these pregnant women. Prenatal care was supervised by a large number of physicians with varying degrees of interest in the obstetric complexities attendant to multiple gestation. In this initial analysis of the data, our attention was focused primarily on the mother and her infants from the point of view of survival.

Subsequently, the data base will be subject to further analysis of other aspects of multiple gestation, such as the interaction between medical complications of pregnancy and methods of management of labor and delivery with the birth weight and weight-specific twin mortality rates. To maintain a proper perspective of future improvements in fetal survival which might occur in the future, the present monitoring system for twin deliveries will be continued in this group of hospitals through 1980, at which time the 1976 to 1980 experience will be reported and compared with the present data.

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Discussion

Editors' note: This manuscript was revised after this discussion was presented.

DR. MARTIN N. MOTEW, Chicago, Illinois. Keith and associates, in a well-organized retrospective study of the outcome of twin pregnancies from 1971 to 1975, set the data base for comparison of subsequent twin pregnancy analysis in a similar population through 1980, the establishment of a level III perinatal service and regionalized care being one of the major differences between the two comparative groups.

Their analysis of the demographic data describes the studied population accurately. It would, however, be important in comparing future study populations to know not only the ethnic distribution of the population but also the twinning incidence and mortality rates in each ethnic group, in case the distribution of the different groups changes. In order to analyze the mortality rates in twins, independent of general changes which also affect singleton pregnancies, analysis of the singleton population at the time of each study is important. The perinatal mortality rate of 6.6% in twin deliveries compares favorably to that of other studies. Naeye and associates,[1] in a recent comparative study in America and Africa, found a 13.9% twin perinatal mortality rate as compared to a 3.3% rate for singleton deliveries. The singleton mortality rate in Dr. Keith's study is not mentioned in the article, thus making it hard to compare groups.

Prematurity of twin pregnancies in this study (33%) and late detection (33%) after the onset of labor are risk factors, as mentioned in the paper, that are hard to control. Improved diagnostic techniques and treatments may, however, lower the risk factors. Persson and colleagues,[2] in an excellent paper on improved outcome of twin pregnancies, showed that by making an early diagnosis of twins by routine ultrasound screening of all antenatal patients at 19 weeks' gestation, and then hospitalizing those patients with twins from the twenty-eighth to the thirty-sixth week of gestation, reduction of the perinatal mortality rate to that of singleton pregnancies was accomplished. Correlation of mortality rates and gestational age at time of discovery of the twin pregnancy would be an important evaluation in determining if future improved diagnostic and therapeutic efforts are successful in preventing premature delivery, the most important factor in the increased perinatal mortality rate.

As mentioned by the authors, bed rest with hospitalization is a controversial treatment. Powers and Miller[3] reviewed twin pregnancies in the North Central Illinois Perinatal Region as regards the influence of bed rest during the most vulnerable period of gestation, 27 to 34 weeks. The cost of hospitalization was approximately 4.5 times the cost of the care of infants who were delivered prematurely and were treated in the Intensive Care Nursery. Other studies[4-6] have shown decreased mortality rates with increased weights at the time of delivery for patients put at bed rest. Other authors, such as Jonas[7] and Harris and Gibbs,[8] noted no change with this treatment.

Medearis and associates,[9] in a 5-year analysis of statewide statistics in Missouri, studied the effects of various complications such as pre-eclampsia, abruptio placentae, premature rupture of membranes, maternal diabetes, and Rh incompatibility on twin mortality rates. They also analyzed the effects of delivery of twins by cesarean section and found no significant difference in mortality rates when compared to vaginal deliveries. These factors should be analyzed in comparing the two groups to be studied.

The authors' Table VI includes data concerning the outcome of the second twin. According to many studies, this depends on such variables as method of delivery, anesthetics used, operative procedures performed, and time interval between delivery of the first and second twin. It would also be helpful to analyze the route of delivery of the second twin, as this seems to be changing the mortality rates of multiple pregnancies. Of particular note in this regard is the difference in death rate between twin 1 and twin 2, each weighing 1,000 to 1,499 gm (35/100 versus 77/100). An analysis as to why such a high mortality rate occurred in this range for the second twin, despite the same facilities being available for both infants, might be vital in trying to compare the next 5-year study with the first.

Another area not analyzed in this study which might alter therapy or care is the incidence of monozygosity in those infants who died (mortality rate being 2.5 times that of dizygotic twins). This factor can now be determined antenatally with ultrasound (demonstration of twin sacs). The risk of interlocking twins can be prevented, fetus-fetus transfusion syndrome can be diagnosed, and disproportionate twins can be followed up antenatally and appropriate measures taken. Robinson and Caines[10] noted that twinning with subsequent degeneration of one fetus occurs at a much higher rate than ever suspected, as demonstrated by ultrasound.

The authors clearly state that factors such as early diagnosis, inhibition of premature labor, ultimate increasing of birth weight, and improved treatment care facilities for the neonate will decrease mortality rates. They also note other critical factors that will need to be studied and compared. These include management of the second twin, evaluating the routes of delivery, zygosity, and the effects of having ultrasonography to diagnose and follow up these high-risk fetuses.

The next 5-year study, which will be prospective and based on this first study, will be even more rewarding in helping us to analyze not only the effect of regionalization of perinatal care but also the modernization of techniques in caring for the twin gestation.

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Detection of gestational diabetes by means of ultrasonic diagnosis of excessive fetal growth

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Excessive abdominal transverse fetal diameter is demonstrated to be a sensitive screening factor in the selection of gestational diabetic patients. An oral glucose tolerance test was performed in 92 pregnant women with potential gestational diabetes criteria and in 21 women without potential diabetic characteristics but with an abdominal transverse fetal diameter above the ninety-fifth percentile. Thirty-eight percent of the women in the latter group had an abnormal oral glucose tolerance test. Moreover, most infants born to gestational diabetic women had an excessive abdominal transverse diameter although the biparietal diameter and birth weight remained fairly normal. (AM. J. OBSTET. GYNECOL. 138:790, 1980.)

THE EFFECT of maternal diabetes on fetal macrosomia is well known, although the physiopathologic mechanisms involved have not yet been clearly elucidated. Ultrasonic survey allows recognition of large-for-dates fetuses before delivery and has permitted us to diagnose gestational diabetes in some apparently healthy pregnant women with only abnormal fetal measurements. As a result of these findings, we were interested in a retrospective study on the relationship between gestational diabetes and ultrasonic fetal measurements.

Patients and methods

Glucose tolerance was investigated in 113 pregnant women without earlier known diabetes, after the thirty-second week of pregnancy, between January 1, 1978, and April 30, 1979. Ninety-two patients were potentially diabetic, with one or more of the following characteristics: diabetes in the close family, obesity, previous child with birth weight over 4,500 gm or unexplained stillbirth, fasting glycosuria, or hydramnios. Twenty-one were studied because of an ultrasonic measurement of a fetal abdominal transverse diameter greater than the ninety-fifth percentile of our own curves, without any other indications of potential diabetes.

An oral glucose tolerance test (OGTT) was performed in the morning, with the nonsmoking patient sitting at rest after a normal food intake for 72 hours and fasting at least the previous 10 hours. The glucose intake was 1 gm/kg of body weight. The plasma glucose level was determined on venous blood and measured by the automatic colorimetric enzymatic method. According to Pedersen,[1] the OGTT was considered as abnormal if the values at 120 and 150 minutes, respectively, were 167 mg/100 ml (9.3 mmoles/L) and 145 mg/100 ml (8.0 mmoles/L) or higher. These plasma glucose values correspond to 140 mg/100 ml (7.6 mmoles/L) and 120 mg/100 ml (6.5 mmoles/L) of whole blood.[2]

Ultrasonic measurements were performed by means of a real-time scanner (2.25 MHz transducer). Biparietal diameter was measured according to the method of Campbell.[3] The abdominal transverse diameter was measured at right angles to the longitudinal axis at the level of the umbilical vein.[4] Results were interpreted according to curves established in our department (Fig. 1).

Results

OGTT results for potentially diabetic women and women with large fetuses are shown in Table I. The incidence of abnormal OGTTs was 14% in potentially

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Received for publication May 8, 1980.

Revised and accepted August 15, 1980.

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0002-9378/80/230790+03$00.30/0 © 1980 The C. V. Mosby Co.

Table I. OGTT results according to selection criteria

	Women (No.)	*Normal OGTT*	*Abnormal OGTT*
Potentially diabetic	92	79 (86%)	13 (14%)*
Abnormal transverse diameter > ninety-fifth percentile	21	13 (62%)	8 (38%)*

*$p < 0.05$ by chi-square test = 5.0 with Yates correction.

gestational diabetic women and significantly higher (38%) in women with large-for-dates fetuses but without potentially diabetic characteristics. Twelve potentially gestational diabetic women also had an abnormal fetal transverse diameter measurement above the ninety-fifth percentile and four of these women (33%) demonstrated an abnormal OGTT.

Table II depicts the OGTT results in the 113 women studied as a function of abdominal fetal measurement. Women with a fetal abdominal transverse diameter under the ninety-fifth percentile had an abnormal OGTT in only 11% of the cases, while those with a diameter above the ninety-fifth percentile had an abnormal OGTT in 36% of the cases.

Of all the patients studied, abnormal OGTTs were found in 21 cases. Fetal measurements in these cases are shown in Table III. The fetal abdominal transverse diameter was above the ninety-fifth percentile in 12 cases (57%) while fetal biparietal diameter was above the ninety-fifth percentile in only 6 cases (28.5%).

All but one of the infants were healthy at birth and during the first 6 days. One fetus died 2 days before birth; the mother was obese, with previously known hypertension, and had a normal OGTT.

Among the 21 infants of mothers with gestational diabetes, three had a birth weight above the ninetieth percentile and only one of them was above the ninety-fifth percentile. The mean birth weight of these infants was 3,460 gm (SD = 415 gm) and the mean duration of gestation was 38.2 weeks (SD = 1.2 weeks). It must be pointed out that all gestational diabetic patients had a restricted diet of 1,600 to 2,000 calories with 40% carbohydrates, according to their weight.

Comment

Infants who are overweight for gestational age have been recognized for a long time as characteristic of the infant born to a diabetic woman. In our series only one infant of the 21 born to gestational diabetic women had birth weight over the ninety-fifth percentile and the mean birth weight of these infants shows only a small excess when compared with the mean birth weight of infants of the same duration of gestation in our hospital (3,250 gm at week 38). This may be related to strict blood sugar control as suggested by other authors.[1, 5]

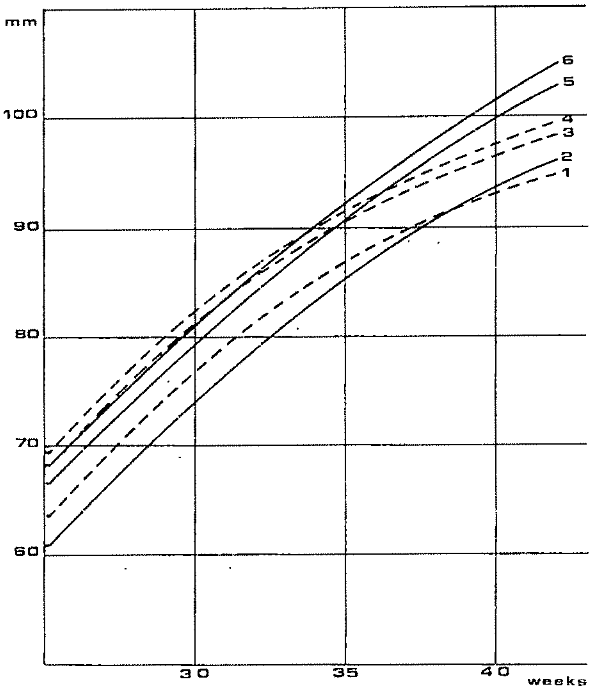

Fig. 1. Reference curves for fetal ultrasonic measurements. Biparietal diameters for the fiftieth *(1)*, ninetieth *(3)*, and ninety-fifth *(4)* percentiles and abdominal transverse diameters for the fiftieth *(2)*, ninetieth *(5)*, and ninety-fifth *(6)* percentiles as shown. The means and percentile limits were established for each week of pregnancy, from 25 weeks to term (40 to 166 patients for each week; normal distribution of data with range of SDs between 2.6 and 3.8 mm for biparietal diameter and between 4.1 and 6.0 for abdominal transverse diameter). In total, 1,817 individual measurements of both parameters were obtained from patients with singleton pregnancies and fetal birth weights between the fifth and the ninety-fifth percentiles.

Diagnostic ultrasound is a useful tool to recognize abnormal fetal growth patterns prior to birth. Fetal head measurement, especially the biparietal diameter, is the most widely used parameter but it does not give the best results in estimation of birth weight. Campbell and Wilkin[4] reported the use of fetal abdominometry for the prediction of fetal weight with improved accuracy. Wladimiroff and co-workers[6] have shown that the ultrasonic measurement of the fetal chest area is superior to the biparietal diameter measurement for the diagnosis of large-for-dates infants. Our results show that the abdominal transverse diameter is above the ninety-fifth percentile in 57% of the infants of gestational diabetic women while the biparietal diameter remains under the ninety-fifth percentile in most of the cases. Murata and Martin[7] also found a normal biparietal diameter in most infants of diabetic mothers.

Table II. OGTT results according to fetal abdominal transverse diameter measurements

Abdominal transverse measurement	*Normal OGTT*		*Abnormal OGTT*	
≤ Nintieth percentile	(90%) 60	71 (89%)	(10%) 7	8 (11%)*
> Nintieth and ≤ ninety-fifth percentile	(85%) 11		(15%) 2	
> Ninety-fifth percentile		21 (64%)		12 (36%)*

*$p < 0.01$ with chi-square test = 9.04.

Table III. Ultrasonic measurements in 21 cases of gestational diabetes

Measurement	*Biparietal diameter*	*Abdominal transverse diameter*
≤ Ninetieth percentile	12 (57%)	7 (33.5%)
> Ninetieth and ≤ ninety-fifth percentile	3 (14.5%)	2 (9.5%)
> Ninety-fifth percentile	6 (28.5%)*	12 (57%)*
Total	21 (100%)	21 (100%)

*$p < 0.05$ with paired series test.

Apparent discordance in the effects of diabetes on cephalic and abdominal measurements may be explained by anatomic findings. It is now well established that macrosomia of the infant of a diabetic mother is principally the result of excessive fat storage.[8] In addition, there is an enlargement of fetal organs which affects generally the pancreas, the heart, and often the liver.[9] All these effects may be responsible for the enlargement of the abdomen. On the contrary, the brain of the infant of a diabetic woman is often smaller than that in a normal infant.[9]

From a diagnostic viewpoint an enlarged abdominal diameter has been found to be a worthwhile indicator of potential gestational diabetes. Thirty-eight percent of the women with only this anomaly had an abnormal OGTT. These results agree with those of Sutherland and Stowers,[10] who found a great number of abnormal intravenous glucose tolerance tests when an excessive fetal fat outline on a radiograph was present either alone or associated with other criteria of potential gestational diabetes. The ultrasonic measurement of the abdominal diameter of the fetus can, therefore, improve the detection of gestational diabetes. The influence of unrecognized gestational diabetes upon the perinatal mortality rate is well documented.[1, 11-14] Most authors emphasize the necessity of improved screening and identification of gestational diabetes. An excessive fetal abdominal size shown by an ultrasound survey seems to be a useful addition to the classic criteria of potential gestational diabetes.

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Transplacental ampicillin: Inhibitory concentrations in neonatal serum

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Concentrations of ampicillin were measured in maternal serum at delivery, in cord serum, and in neonatal serum sampled at 1, 4, and 8 hours after ampicillin had been administered prophylactically to 22 mothers who were undergoing cesarean section. The concentrations of ampicillin in maternal serum at delivery ranged from 4.6 to 60 μg per milliliter and were inversely related to the time between administration of the drug and delivery. Concentrations in cord serum ranged from 4.4 to 23 μg/ml and were lower than those in corresponding maternal samples in all but two cases. The ratios of cord/maternal concentrations of ampicillin ranged from 0.16 to 1.3 and were directly related to the time which had elapsed between administration of ampicillin and delivery. The mean concentration of ampicillin in cord and neonatal serum declined exponentially in the 8 hours after infusion; it exceeded the minimal inhibitory concentration against *Escherichia coli* for 4 hours and against *Listeria monocytogenes* and group B streptococcus for at least 8 hours. (AM. J. OBSTET. GYNECOL. 138:793, 1980.)

POSTPARTUM FEBRILE MORBIDITY is a frequent complication of cesarean section and significantly increases the length and cost of hospitalization for affected mothers. Many studies have demonstrated the effectiveness of prophylactic preoperative administration of antibiotics in reducing the incidence of this complication,[1-4] especially in mothers classified as being at high risk.[5] Ampicillin, because of its broad spectrum, relative lack of toxicity to mother and fetus, and capacity to achieve high levels in serum, is one of the antibiotics most frequently used for this purpose. Several investigators have demonstrated placental transfer of ampicillin to the fetus and amniotic fluid,[6-8] and one study showed the persistence of measurable levels in the infants' sera for 4 hours.[7]

The purpose of this study was to determine whether prenatal administration of ampicillin results in neonatal serum concentrations which are inhibitory for organisms that commonly infect newborn infants, and if so, for how long.

Material and methods

Patient selection and procurement of samples. The study group consisted of 22 mothers, for whom the attending obstetrician prescribed prophylactic ampicillin prior to cesarean section, and their newborn infants, at North Carolina Memorial Hospital during September and November, 1978, and January, 1979. The project was approved by the institution's Committee on the Protection of the Rights of Human Subjects, and informed consent was obtained from the mothers prior to any preoperative medication. Each mother received an intravenous infusion of 1 gm of sodium ampicillin in 250 ml of 5% dextrose over 10 to 20 minutes, begun at times ranging from 10 minutes to 127 minutes prior to delivery.

Maternal blood was obtained at the time of delivery by venipuncture from the arm which had not been used for the infusion of ampicillin. Cord blood was obtained in the routine manner, with no attempt being made to separate umbilical venous and arterial blood. Neonatal blood was obtained by venipuncture, heelstick, or via umbilical artery catheter, if one was already

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Supported in part by a grant from North Carolina United Community Services.

Received for publication April 7, 1980.

Accepted July 28, 1980.

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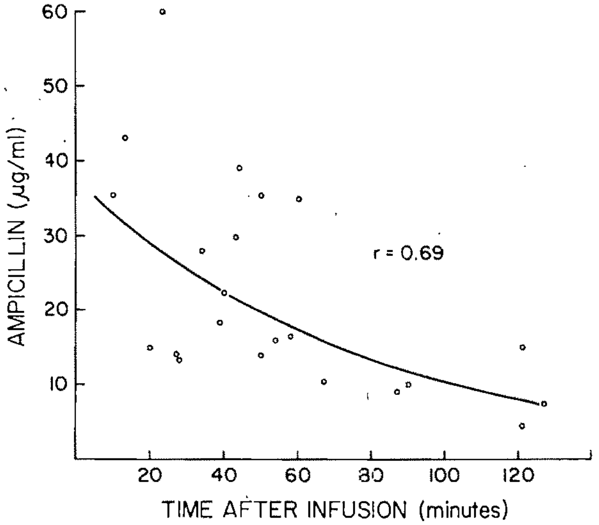

Fig. 1. Concentrations of ampicillin in maternal serum at delivery, in relation to time between infusion and delivery.

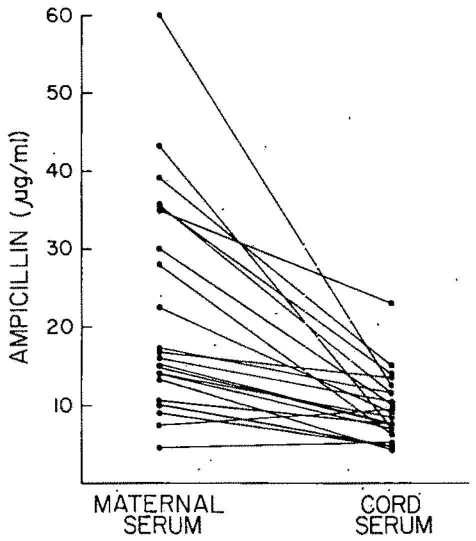

Fig. 2. Concentrations of ampicillin in maternal serum at delivery and in corresponding samples of cord serum.

in place for another reason, at approximately 1, 4, and 8 hours after the start of the maternal infusion of ampicillin; the exact times were recorded. Samples were allowed to clot at room temperature and were centrifuged; serum was removed and stored at −70° C for less than 24 hours, prior to assay.

Three infants were suspected of sepsis and received infusions of ampicillin between 1 and 8 hours of age. Subsequent samples from these infants were not included in the data. Complete data (all five samples) are reported for 14 mother-infant pairs, at least four samples from 17 mother-infant pairs, and at least three samples from all 22 mother-infant pairs.

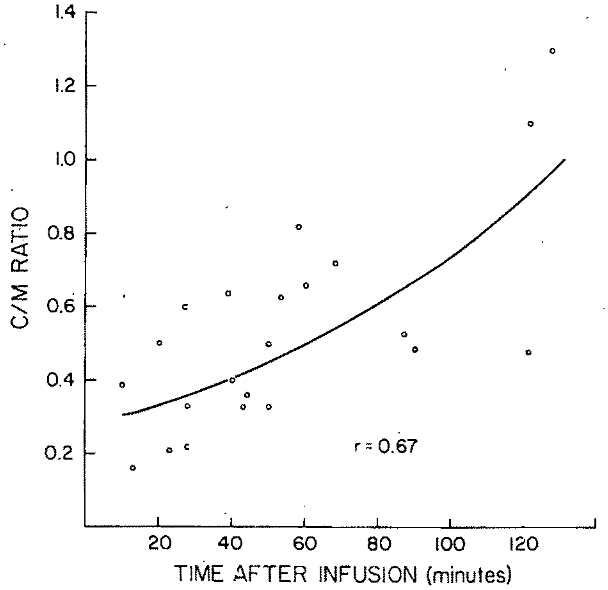

Fig. 3. Relationship of cord/maternal *(C/M)* serum ampicillin concentration ratios to time between administration of ampicillin and delivery.

Assay of ampicillin in serum specimens. A modification of the bioassay method described by Bennett and associates[9] was used to measure the concentrations of ampicillin in all specimens of serum. One milliliter of a 1:10 dilution of a *Bacillus subtilis* ATCC 6633 spore suspension (Difco Laboratories, Detroit, Michigan) was added to 70 ml of molten sterile trypticase soy agar that had been cooled to 55° C. After thorough mixing, the agar-spore mixture was dispensed into a 150 by 15 mm sterile Petri dish. The agar was then allowed to solidify on a level surface.

As many as 18 wells were created in each solidified agar plate through use of a sterile No. 2 cork borer. The agar plugs were aspirated by a vacuum trap apparatus.

Ampicillin powder of known potency provided by Beecham Laboratories (Bristol, Tennessee) was used to prepare antibiotic standards in pooled normal human serum (Gibco Diagnostics, Grand Island, New York). The concentrations of ampicillin employed for these standards were: 10, 5, 2.5, and 1.25 μg/ml. Serum assays were performed by adding 50 μl duplicate samples of standards and undiluted serum specimens, as well as 1:2, 1:4, and 1:6 dilutions of serum specimens, to individual wells of the spore-containing plates. Assay plates were then incubated at 35° C for 18 hours on a level surface. After incubation, the zone of inhibition surrounding each well was measured with a caliper. A standard curve was constructed by plotting the mean zone size for each concentration of standard versus the concentration of ampicillin. The concentration of ampicillin in each of the patient specimens was deter-

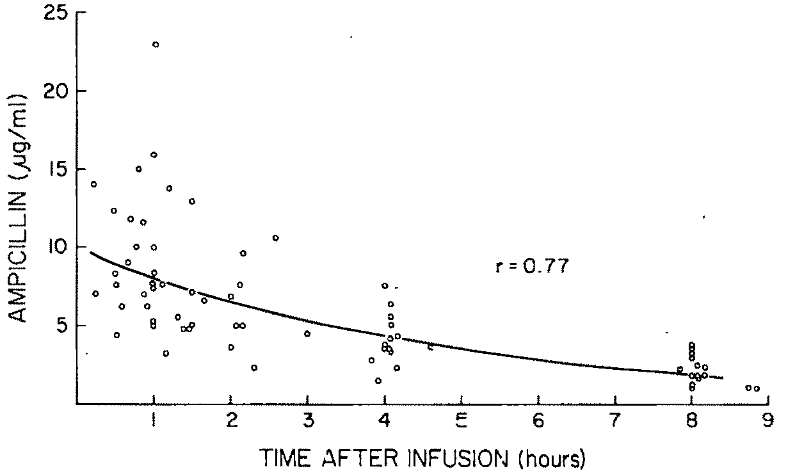

Fig. 4. Mean disappearance curve for ampicillin in cord and neonatal serum after maternal infusion of ampicillin.

mined by relating the average zone size to the standard curve and determining the corresponding concentration of ampicillin.

On each day of testing, duplicate samples that contained varying known concentrations of ampicillin were also tested. If the concentration of ampicillin for one of these samples exceeded a ± 10% variation from that which was known, all samples tested on that day were retested. The assay was found to be capable of measuring ampicillin with an accuracy of ± 10%.

Minimal inhibitory concentrations. A review was made of the results of dilution susceptibility tests performed with ampicillin upon bacteria recovered from either the blood or cerebrospinal fluid of infants less than 1 month of age who were cared for in this institution between October, 1976, and July, 1979. Minimal inhibitory concentrations (MICs) were determined for blood or cerebrospinal fluid isolates of *Listeria monocytogenes* and *Escherchia coli* by means of a broth dilution method that adhered to the recommendations of an international collaborative study.[10] MICs for *Haemophilus influenzae* were determined by means of a microtiter broth dilution method.[11]

Statistical methods. All concentrations of ampicillin in cord and neonatal serum samples were plotted against time elapsed from maternal injection. The mean ampicillin disappearance curve was determined from a series of computerized programs for "best fit."

Results

The infant population was comprised of four preterm infants (33, 30, 28, and 36 weeks), 17 term infants, and one postterm infant (43 weeks). Birth weights ranged from 940 to 4,000 grams. Three infants were small for gestational age, and the other 19 were of appropriate weight for gestational age. Five of the infants were admitted to the neonatal intensive care unit for prematurity and/or illness in the neonatal period. One of these infants died of prematurity, respiratory distress syndrome, and intraventricular hemorrhage. None of the infants had documented bacterial infection during the neonatal period.

Concentrations of ampicillin in cord serum and the ampicillin disappearance curves in neonatal serum were not significantly different for the four premature infants when compared with those of the 18 term and postterm infants, nor for the three ill newborn infants when compare with the 19 well infants. Therefore, all data were pooled for analysis.

The concentrations of ampicillin in maternal serum at delivery ranged from 4.6 to 60 μg/ml and were inversely related to the time between administration of ampicillin and delivery (Fig. 1). The concentrations of ampicillin in samples of cord serum ranged from 4.4 to 23 μg/ml and were lower than those in corresponding maternal samples in all but two cases (Fig. 2). The ratios of cord to maternal concentrations of ampicillin (C/M ratios) ranged from 0.16 to 1.3 and were directly related to the time which had elapsed between administration of ampicillin and delivery (Fig. 3). Concentrations of ampicillin in neonatal serum declined exponentially in the 8 hours subsequent to maternal administration (Fig. 4).

Among the bacterial isolates encountered in specimens of blood or cerebrospinal fluid from neonates in North Carolina Memorial Hospital during the 34-month period under review, there were four strains of group B streptococci, three strains of *Listeria monocytogenes*, and one strain each of β-lactamase negative *Haemophilus influenzae, Escherichia coli,* and *Streptococcus* *faecalis* for which ampicillin MICs had been determined. With the exception of the single isolate of *E. coli*, all organisms demonstrated ampicillin MICs of less than 1 μg/ml. MICs for group B streptococci ranged between 0.06 and 0.25 μg/ml. The single *E. coli* strain tested yielded an ampicillin MIC of 4.0 μg/ml. Four other *E. coli* strains recovered from cultures of blood or cerebrospinal of neonates during the same period were susceptible to ampicillin when tested by the disc diffusion method.

Comment

We have shown mean concentrations of ampicillin in the infants' sera which exceeded the minimal inhibitory concentration for 4 hours against the less sensitive *(E. coli)* and for at least 8 hours against the more sensitive (*L. monocytogenes* and group B streptococcus) organisms which commonly cause neonatal infections. The concentrations of ampicillin in cord sera in all cases exceeded minimal inhibitory concentrations for ampicillin susceptible organisms which cause neonatal sepsis in this hospital. Ampicillin susceptibility of bacteria encountered in this institution is similar to that reported by others.[12, 13]

The longer the interval between a single maternal dose and delivery, the larger the ratio of cord to maternal serum concentrations at delivery. Infants born more than 2 hours after maternal administration of ampicillin may have serum concentrations of ampicillin equal to or greater than those of their mothers. These data confirm those of Bray and associates,[7] who documented the persistence of ampicillin in the neonatal serum for up to 4 hours and found the concentrations in cord serum equal to those in maternal serum 1½ to 2 hours after maternal administration.

At present, no data exist which clearly indicate whether ampicillin acquired placentally just prior to delivery influences the risk of morbidity and mortality due to infection in the newborn infant.

The current widespread practice of prophylactically administering ampicillin just prior to cesarean section may create a dilemma in evaluating the infant for infection. Ampicillin has been shown to prevent group B streptococcal colonization of infants born to previously colonized women who were treated with intravenous ampicillin during labor.[14] However, transplacental ampicillin could obscure infection by preventing bacterial growth in cultures or suppressing physical signs, and could promote colonization with resistant organisms. A solution to this dilemma is suggested by two recent studies which have shown that ampicillin administered to mothers after clamping of the cord reduces maternal risk of postpartum febrile morbidity to a degree similar to that achieved with preoperative administration.[15, 16]

We wish to thank Diane Manne for statistical assistance.

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Human perinatal distribution of butorphanol

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The perinatal distribution of butorphanol was determined in relation to maternal-neonatal transfer and colostrum/milk excretion in obstetric patients. Parenteral butorphanol passed the placental barrier and was found in neonatal cord serum. The mean neonatal serum concentration of butorphanol was not different from the mean maternal serum concentration of butorphanol, following a 1 or 2 mg intramuscular dose. Butorphanol was detected in the milk of lactating women following oral and intramuscular administration. Serum and milk concentrations appeared to be parallel with time. This observation was confirmed by the constancy of the mean milk-to-serum concentration ratio (0.7 intramuscular, 1.9 oral). We calculated that 4 μg would be the maximum amount of butorphanol, which would be expected to be present in the full daily milk output (1 L) following administration four times a day of 2 mg intramuscular or of 8 mg oral doses. An oral dose of 4 μg to an infant weighing 4 kg corresponds to the negligible oral dose of 0.07 mg to a 70 kg adult. The demonstrated safety and efficacy of butorphanol as an obstetric analgesic and the characteristics of the maternal-neonatal transfer and milk excretion are indicative of the potential excellence of this agent for obstetric use. (AM. J. OBSTET. GYNECOL. 138:797, 1980.)

BUTORPHANOL (*l*-N-cyclobutylmethyl-3,14β-dihydroxymorphinan) is a new, potent, totally synthetic agonist-antagonist analgesic,[1] effective both parenterally and orally. In clinical studies parenterally administered butorphanol exhibited an analgesic potency five times greater than that of morphine,[2] 20 times greater than that of pentazocine,[3] and 40 times greater than that of meperidine.[4] Maduska and Hajghassemali[5] demonstrated the safety and efficacy of parenteral butorphanol as a prepartum analgesic. Drug effects in the infant can be related to placental transfer in the neonate[6] and excretion in milk in the nursing mother.[7] The maternal-fetal transfer and colostrum/milk excretion of butorphanol were determined in the present clinical studies in order to establish the perinatal disposition of this drug.

Methods

Maternal-neonatal transfer study. Six healthy patients with moderate to severe prepartum pain (age 21 ± 2 years, height 162 ± 5 cm, and prepartum weight 66 ± 14 kg) participated in this study. The study protocol was approved by an Institutional Review Board, and a signed informed consent was obtained from each subject. Butorphanol tartrate (Stadol) was administered intramuscularly once or twice at doses of 1 or 2 mg at times ranging between 1.4 and 6.3 hours before delivery (Table I). Maternal and neonatal cord blood samples were obtained just after delivery, allowed to clot, and centrifuged to obtain serum. Serum

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Received for publication February 8, 1980.

Revised July 15, 1980.

Accepted July 21, 1980.

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0002-9378/80/230797+04$00.40/0 © 1980 The C. V. Mosby Co.

Table I. Maternal and neonatal serum concentrations of butorphanol following intramuscular administration of butorphanol tartrate

No.	Dose (mg)	Time of sample collection following dose	Serum butorphanol (ng/ml) Maternal	Serum butorphanol (ng/ml) Neonatal	Neonatal: maternal ratio
1	1	1 hr, 25 min*	0.76	0.64	0.84
2	1	2 hr, 16 min*	0.40	0.16	0.40
3	2	2 hr, 58 min*	0.73	1.02	1.40
4	1	2 hr, 27 min*	0.57	0.55	0.96
5	2	6 hr, 21 min*	0.84	0.64	0.76
	2	3 hr, 41 min*			
6	1	3 hr, 26 min*	1.25	0.83	0.66
	1	41 min*			

*The approximate elapsed time between dosing and delivery.

Table II. Mean serum and colostrum/milk concentrations of butorphanol in nursing women following single intramuscular and oral doses of butorphanol tartrate

Dose	Route	Fluid	Butorphanol (ng/ml) 1 hr	1.5 hr	3 hr	4 hr	6 hr	6.5 hr
2 mg	Intramuscular	Serum	2.4 ± 1.3		1.1 ± 0.7		0.5 ± 0.2	
		Colostrum/milk	1.5 ± 0.9		0.7 ± 0.5		0.3 ± 0.2	
		Milk-to-serum ratio	0.7		0.8		0.7	
8 mg	Oral	Serum	2.5 ± 1.2		1.2 ± 1.0		0.6 ± 0.4	
		Colostrum/milk	3.6 ± 2.6		1.8 ± 1.0		1.1 ± 0.6	
		Milk-to-serum ratio	1.7		1.7		2.2	

samples were immediately frozen and stored at −20° C prior to analysis. Butorphanol was stable in serum under these storage conditions.

Colostrum/milk excretion study. Twelve healthy, lactating, postpartum women (age 27 ± 7 years, height 158 ± 4 cm, and prepartum weight 65 ± 9 kg) participated in this study. The study protocol was approved by an Institutional Review Board, and a signed informed consent was obtained from each subject. The subjects were not nursing their infants at the time of study entry and/or agreed to delay breast-feeding until 24 hours after the butorphanol dose. Butorphanol tartrate was administered to six women at a single 2 mg intramuscular dose at 79 ± 20 hours after delivery and to six other women at a single 8 mg (two 4 mg tablets) oral dose at 68 ± 36 hours after delivery.

Predose blood and colostrum/milk samples were obtained just prior to administration of the drug. The breasts were emptied at this time. Blood samples subsequently were collected at 1, 3, and 6 hours following intramuscular administration and at 1.5, 4 and 6.5 hours following oral administration and processed for serum. Colostrum/milk samples were collected at intervals of 0 to 2, 2 to 4, and 4 to 8 hours after intramuscular administration and at intervals of 0 to 3, 3 to 5, and 5 to 8 hours after oral administration, by emptying both breasts at the end of each collection interval. Samples were frozen immediately and stored at −20° C prior to analysis. Butorphanol was stable in these fluids under the storage conditions. The pH of each colostrum/milk sample was determined with a Corning Model 10 pH meter equipped with a silver/silver chloride combination electrode.

Butorphanol analysis. The concentration of butorphanol in serum and milk was determined by a specific radioimmunoassay[8] (RIA) with ^{125}I-butorphanol used as the radiolabeled antigen. The antibody does not cross react with two known metabolites (hydroxybutorphanol and norbutorphanol) but does react with a butorphanol conjugate.[8] The procedure involved extraction of the biological fluid at alkaline pH with hexane/2% isoamly alcohol followed by assay of the reconstituted extract. In the case of colostrum/milk, samples were washed by hexane extraction to remove neutral lipids prior to alkalinization. The RIA procedure was specific for butorphanol since extraction eliminated interference from cross-reacting metabolite(s). The precision of the assay was 6% to 9% and the accuracy was 8% to 12%.[8]

Statistical analyses. The mean maternal and neonatal serum concentrations were compared by means of the paired t test. The mean milk-to-serum concentration ratios for each collection period with each route of administration were compared with a two-way analysis of variance (ANOVA).[9] The overall mean milk-to-serum concentration ratio after intramuscular administration was compared to the mean ratio after oral administration, with the t test[9] on subject means taken across time. The variability of all means in this report is represented as the mean ± standard deviation.

Results

Butorphanol passed the placental barrier and was distributed into the neonatal serum. Maternal and neonatal serum butorphanol concentrations are presented in Table I. The mean maternal serum–to–neonatal serum ratio was 0.9, which was not significantly different than unity, since the mean difference between maternal and neonatal serum was not significant ($p > 0.05$, t test).

Butorphanol was detected in milk following both intramuscular and oral administration. Serum and milk concentrations of butorphanol are presented in Table II. A graphic representation of mean serum and milk concentrations is shown in Fig. 1. Serum and milk concentrations were higher after the 8 mg oral dose than after the 2 mg intramuscular dose. The apparent parallelism of the serum and milk concentrations with time was confirmed by the constancy of the mean milk–to–mean serum concentration ratio (Table II). No significant differences ($p > 0.05$, ANOVA) between mean ratios were observed at any time after either intramuscular or oral administration. However, the overall mean ratio after intramuscular administration (0.7) was significantly ($p > 0.001$, t test) less than the ratio (1.9) after oral administration. The mean pH of colostrum/milk from the women receiving the intramuscular dose was 7.1 ± 0.2 (SD) (N = 24) and that from the women receiving the oral dose was 7.1 ± 0.3 (SD) (N = 24).

No depression was observed in newborn infants following administration of butorphanol to the mothers just prior to or after parturition.

Comment

The passive transport of xenobiotics and endogenous chemicals across the placenta is regulated by the physicochemical properties of the chemical and the physiologic characteristics of the maternal-placental-fetal unit.[6] Butorphanol, a moderately lipophilic, basic compound with a pKa of 8.6, would exist primarily in the ionized form (94%) at a plasma pH of 7.4. Protein binding can decrease diffusion and lower the amount of fetal drug because of lower protein binding in fetal serum. The distribution of butorphanol should not be affected by protein binding, since it is only 80% bound to plasma proteins.[10] The mean neonatal-to-maternal ratio of serum butorphanol of near unity is characteristic of many basic drugs. A maternal-neonatal ratio of 0.89 has been reported for meperidine[5] and a ratio of 0.6 has been reported for pentazocine.[11]

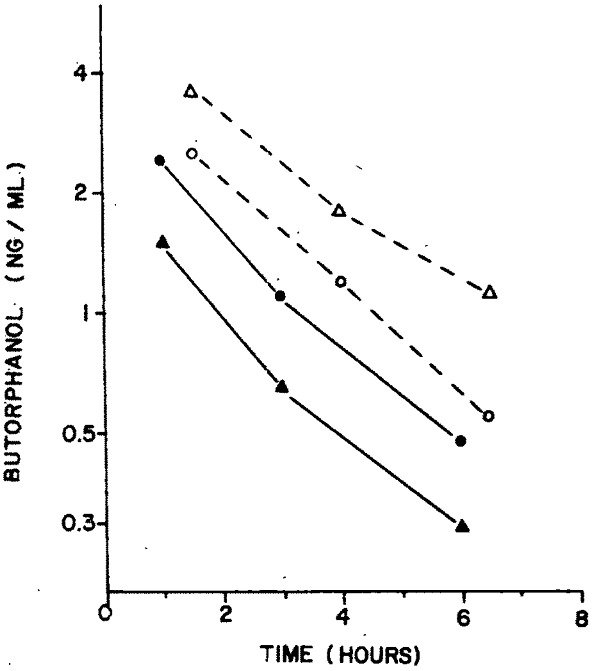

Fig. 1. Semilog plot of mean serum and colostrum/milk concentrations of butorphanol after intramuscular and oral administration of butorphanol tartrate. ● = Serum, 2 mg intramuscularly, N = 6; ▲ = colostrum/milk, 2 mg intramuscularly, N = 6; ○ = serum, 8 mg orally, N = 6; △ = colostrum/ milk, 8 mg orally, N = 6.

Studies[10] with ^3H-butorphanol in normal, adult, male subjects have demonstrated the presence of a major metabolite, hydroxybutorphanol (3,14 dihydroxy-N-(trans-3'-hydroxycyclobutylmethyl) morphinan), in serum and urine following oral and parenteral administration. Norbutorphanol (3,14-dihydroxymorphinan) was identified as a minor urinary metabolite. These metabolites had no activity in the mouse writhing assay at a subcutaneous dose of 40 mg/kg, whereas the ED_{50} of butorphanol was 0.05 mg/kg.[14] Butorphanol metabolites could exist in the fetus as a result of placental or fetal metabolism,[12, 13] but the circulating metabolites of butorphanol, hydroxybutorphanol and butorphanol glucuronide,[8] would not be expected to diffuse efficiently into the fetus, because of their decreased lipophilic activity. Although butorphanol metabolites appear to be devoid of significant pharmacologic activity in animal tests, meperidine metabolites have been implicated in psychophysiologic disturbances and in respiratory depression in the infant.[15] The incidence of neonatal depression was related to the time of injection before delivery and to the dose and the rate of maternal metabolism of meperidine. It was concluded that the metabolites of meperidine, rather than the parent compound, were the probable cause of the depression. The lack of pharmacologic activity of the major metabolites of butorphanol and the demonstration of the clinical safety of butorphanol in the neonate[5] are indicative of the potential superiority of butorphanol as an obstetric analgesic.

There is general agreement that drugs which can be administered to the pregnant mother without risk to the fetus can be given to the nursing mother with assurance of safety for the infant. Nevertheless, a knowledge of the actual extent of excretion of a drug in milk is a critical factor in the assessment of drug exposure in the breast-fed baby.[16] Drug excretion in milk is regulated by the same physicochemical properties which regulate the passive transport process in the maternal-fetal unit.[7] Based upon a pKa for butorphanol of 8.6, a serum pH of 7.4, and the observed mean colostrum/milk pH of 7.1, the theoretical concentration ratio of butorphanol between milk and plasma is 1.9. This value is identical to the mean ratio of 1.9 observed following oral butorphanol. Why the colostrum/milk to serum ratio of butorphanol following intramuscular administration (0.7) should have been different is inexplicable at this time and would be an interesting question for further research. If butorphanol was administered at a 2 mg intramuscular dose or an 8 mg oral dose four times daily, the mean daily serum concentration would be 2.0 ng/ml or less. Based on a mean serum concentration of 2 ng/ml, a daily milk excretion of 1,000 ml, and a milk-to-serum ratio of 2, the maximum daily dose of butorphanol to a neonate would be 4 μg. This corresponds to a negligible oral dose of 0.07 mg to a 70 kg adult. The absence of deleterious effects in the neonate when parenteral butorphanol is used as an obstetric analgesic also indicates safety. The potential for a neonatal effect from the administration of parenteral or oral butorphanol to the nursing mother would appear to be minimal.

We thank B. J. Floor and R. C. Van Buskirk for RIA analysis.

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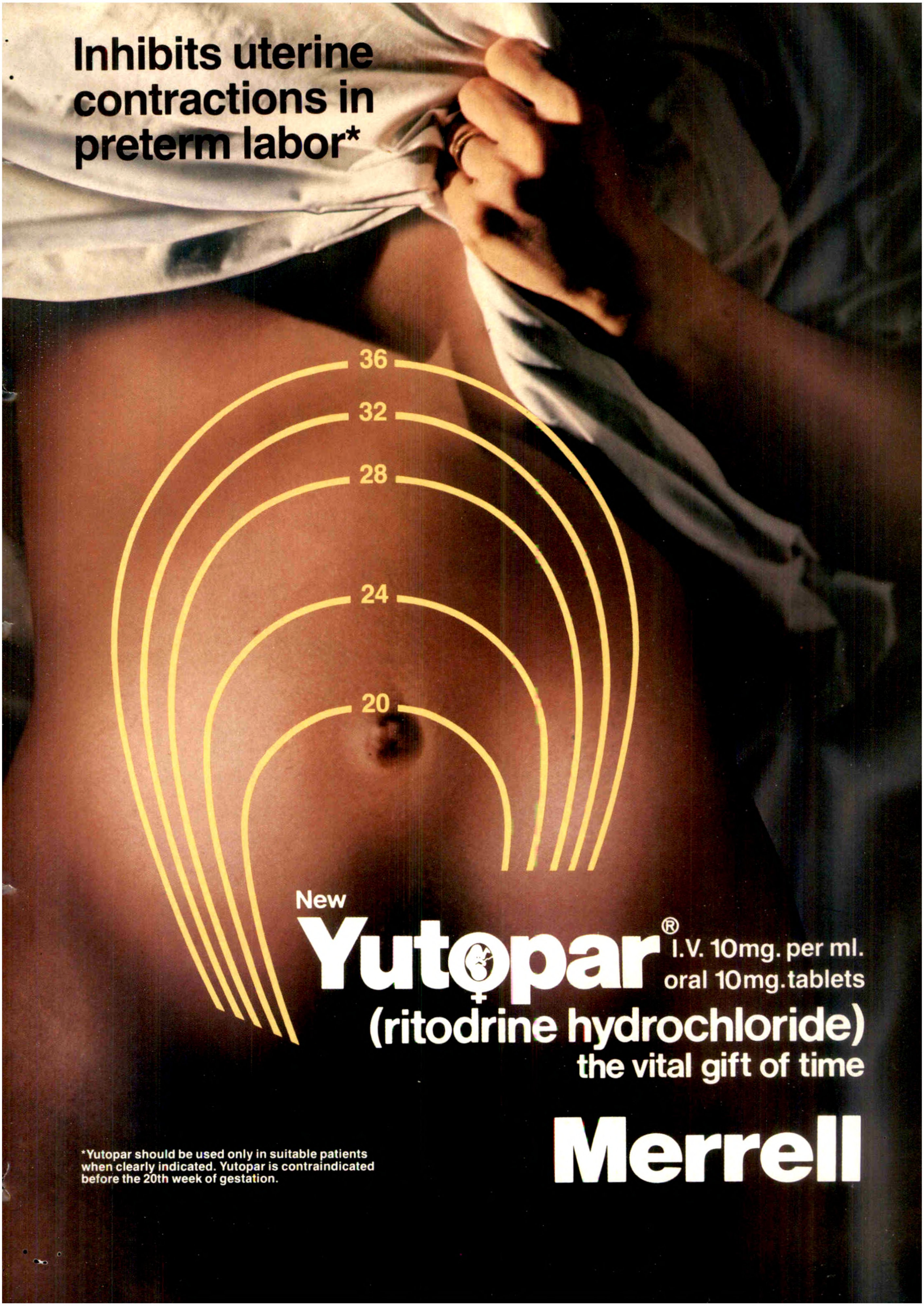


Inhibits uterine contractions in preterm labor*

New

Yutopar® I.V. 10mg. per ml.
oral 10mg. tablets

(ritodrine hydrochloride)

the vital gift of time

Merrell

*Yutopar should be used only in suitable patients when clearly indicated. Yutopar is contraindicated before the 20th week of gestation.

Yutopar®
(ritodrine hydrochloride)

The Vital Gift of Time in Preterm Labor in Suitable Patients

A multicenter series of randomized, prospective, double-blind, controlled studies comparing ritodrine hydrochloride with either ethanol or placebo in the treatment of idiopathic preterm labor have established Yutopar as the first effective tocolytic with generally acceptable side effects available in the U.S.[1] Yutopar is truly the gift of time.

	Ritodrine	**Control**	**Significance**
Time *in utero*			
Mean, days gained	**32.6**	**21.3**	**$p<0.001$**
Gestational Age at delivery (weeks)	**35.5**	**34.5**	**$p<0.05$**
Time to mature			
Incidence of respiratory distress syndrome*	**11%**	**20%**	**$p<0.05$**
Time to be born†			
Attaining 36th week of gestation or beyond	**52%**	**38%**	**$p<0.05$**
Attaining birth weight of 2500 g or more	**58%**	**43%**	**$p<0.05$**
Incidence of neonatal death	**5%**	**13%**	**$p<0.05$**

*When steroids were administered concomitantly to mothers to hasten fetal lung maturity, maternal pulmonary edema occurred in a few cases. (See Warnings.)

†See pregnancy statement in Precaution section of prescribing information.

Merrell

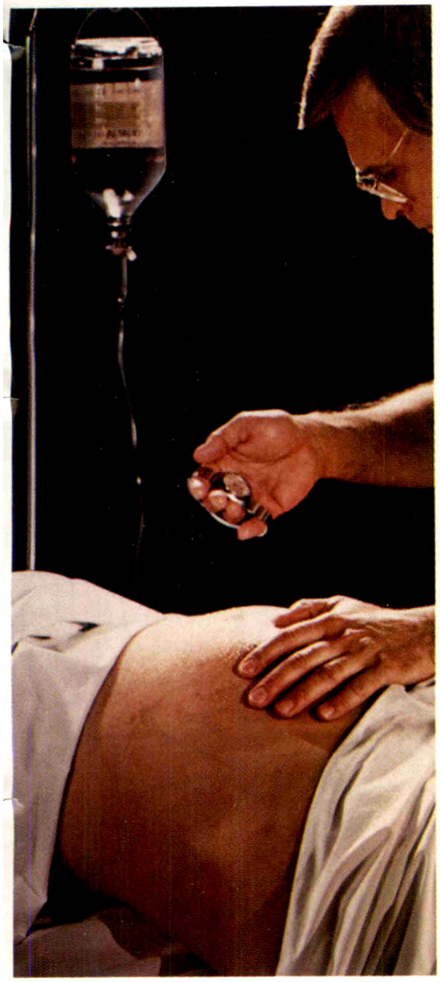
(Please read the Product Information on the last page before prescribing Yutopar.)

The first product indicated exclusively for the management of preterm labor in suitable patients

Clinical Pharmacology

• Uterine effects:

Yutopar is a beta-sympathomimetic agent. Its preferential stimulatory effects are on beta$_2$ receptors, which result in relaxation of uterine muscle.



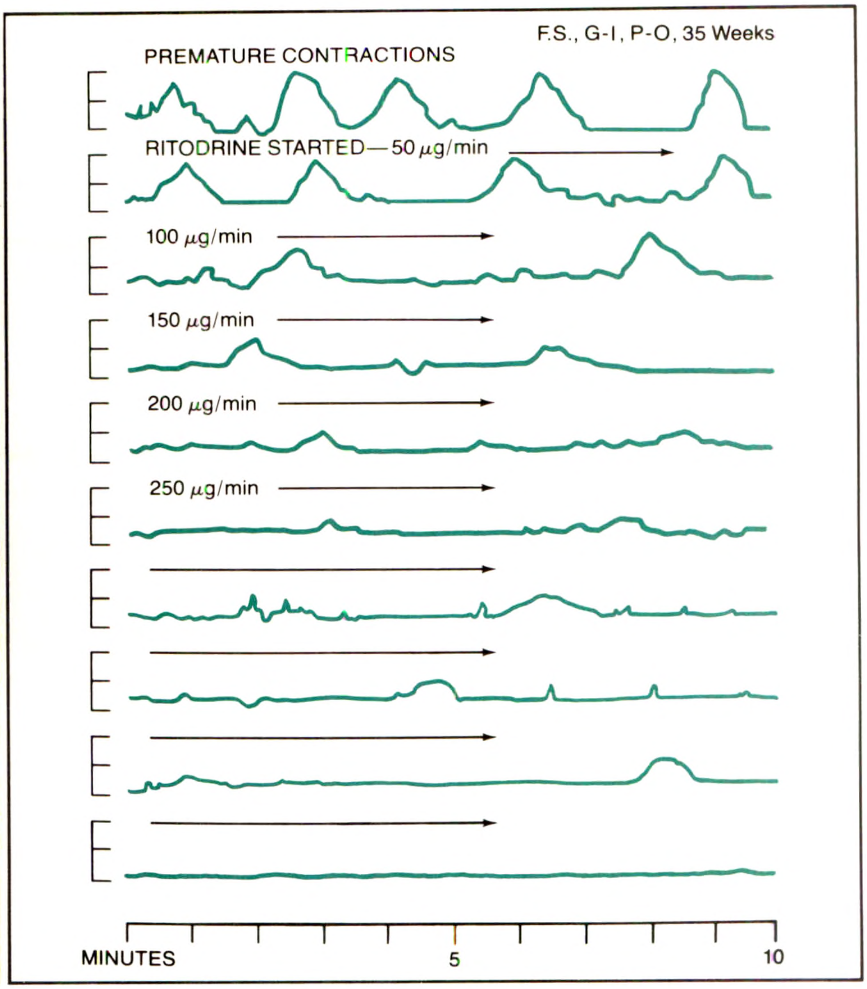


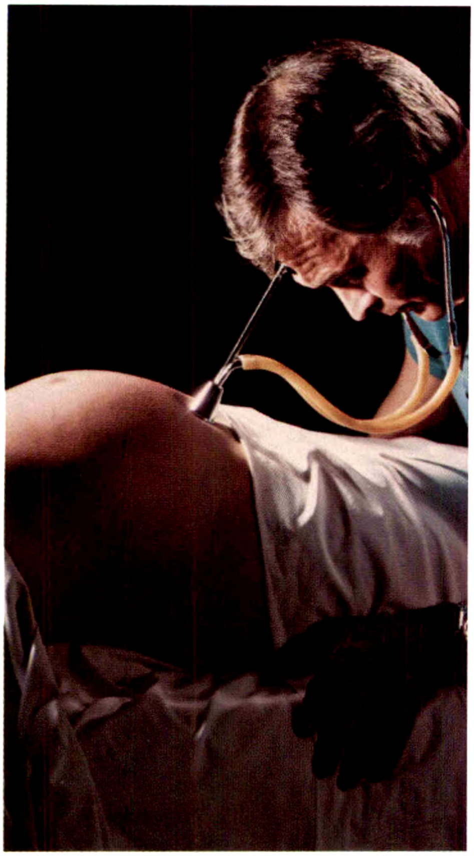
Figure 2. Tracing of the intravenous Yutopar infusion effect on uterine activity from a single patient in preterm labor at 35 weeks. The tracing records, in 10 minute segments monitored by external tocography, uterine activity immediately before and during a 90 minute infusion with the Yutopar dose increasing from 50 to 250 μg. per minute.

From Lauersen *et al.*[1]

Yutopar
the vital gift of time

• Cardiovascular effects:

The effects on the maternal cardiovascular system are dose-related.[3] Marked hypotension is not produced.[3] Cardiac output is increased.[3] Ritodrine passes the placental barrier in sheep[4] and is thought to do so in humans as well. The effects on the human fetal heart rate are modest.[5,6] Because cardiovascular responses are common and most pronounced during intravenous administration of Yutopar, cardiovascular effects, including maternal pulse rate and blood pressure and fetal heart rate, should be closely monitored.



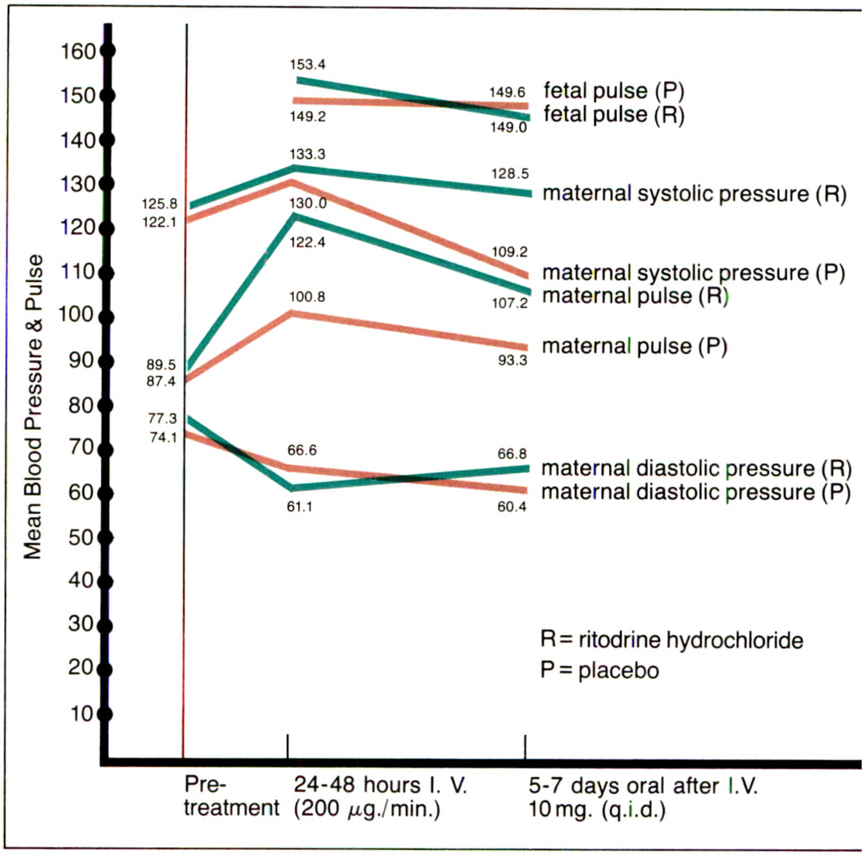

Mean Blood Pressure & Pulse
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
153.4
149.6
fetal pulse (P)
149.2
149.0
fetal pulse (R)
133.3
128.5
maternal systolic pressure (R)
125.8
122.1
130.0
122.4
109.2
maternal systolic pressure (P)
107.2
maternal pulse (R)
100.8
93.3
maternal pulse (P)
89.5
87.4
77.3
74.1
66.6
66.8
maternal diastolic pressure (R)
61.1
60.4
maternal diastolic pressure (P)
R = ritodrine hydrochloride
P = placebo
Pre-treatment
24-48 hours I. V. (200 μg./min.)
5-7 days oral after I.V. 10 mg. (q.i.d.)

Figure 3. The mean maternal pulse pressure and heart rate are shown to increase during intravenous ritodrine treatment and there is a slight rise in the mean systolic blood pressure.[7] These effects are less marked in the control group and show a tendency to subside during oral therapy.

Based on data from Wesselius-DeCasparis *et al.*[7]

• Metabolic effects:

As a consequence of its beta-mimetic mechanism of action, ritodrine intravenously has transient metabolic effects. These may include elevations of blood glucose,[8,9] insulin,[8,9] triglycerides,[10] and free fatty acids.[10] Decreased serum iron and serum potassium have also been found.[10] No effects on other electrolytes have been reported.

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Yutopar
the vital gift of time

(Please read the Product Information on the last page before prescribing Yutopar.)

Yutopar®
(ritodrine hydrochloride)
Dosage and Administration Chart

Frequent monitoring of maternal uterine contractions, heart rate, and blood pressure, and of fetal heart rate is required. The dose of Yutopar should be individually titrated according to response.

INTRAVENOUS

Intravenous administration of Yutopar should be supervised by persons having knowledge of the pharmacology of the drug and qualified to identify and manage complications of drug administration and pregnancy.

The patient receiving intravenous therapy should be kept in the left lateral recumbent position.

Solution: dilute 150 mg. ritodrine hydrochloride (3 ampuls) in 500 ml. solution, which yields a final concentration of 0.3 mg./ml. (Do not use parenteral Yutopar if the solution is discolored or contains any precipitate or particulate matter.) The following solutions may be used for dilution:

- 0.9% w/v sodium chloride
- 5% w/v dextrose
- 10% w/v Dextran 40 in 0.9% w/v sodium chloride
- 10% w/v invert sugar
- Cpd sodium chloride (Ringer's)
- Hartmann's

Method of Administration: a controlled infusion device is recommended to adjust the rate of flow in drops per minute. An i.v. microdrip chamber 60 drops/ml. can provide a convenient range of infusion rates within the recommended dose for Yutopar.

Dosage: the usual initial dose is 0.1 mg./minute, to be increased according to results by 0.05 mg./minute every 10 minutes until the desired result is obtained. The effective dosage usually lies between 0.15 and 0.35 mg./minute. The infusion should generally be continued for at least 12 hours after uterine contractions cease.

Dosage equivalents

(at dilution of 3 ampuls/500 ml. fluid)
if microdrip chamber (60 drops/ml.) is used

mg./min.	equals	drops/min.
.1	=	20
.15	=	30
.2	=	40
.25	=	50
.3	=	60
.35	=	70

Note: At the recommended dilution and rate of administration, the maximum volume of fluid which might be administered over 12 hours at the highest dose (0.35 mg./min.) will be approximately 840 ml. The total amount of i.v. fluids administered and the rate of administration should be monitored to avoid circulatory fluid overload.

Recurrence: may be treated with repeated infusions, as above

ORAL

Maintenance: 1 tablet (10 mg.) approximately 30 minutes before termination of i.v. therapy. For the first 24 hours the usual dosage is 1 tablet every 2 hours. Thereafter, the usual dosage is 1 or 2 tablets (10 to 20 mg.) every 4 to 6 hours, depending on uterine activity and side effects. Total daily dose should not exceed 120 mg.

(Please read the Product Information on the last page before administering Yutopar.)

Merrell

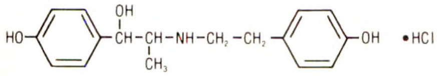
Yutopar®
(ritodrine hydrochloride)
the vital gift of time

CAUTION: Federal law prohibits dispensing without prescription.

DESCRIPTION

Yutopar, which contains the betamimetic (beta sympathomimetic amine) ritodrine hydrochloride, is available in two dosage forms. Yutopar for parenteral (intravenous) use is a clear, colorless, sterile, aqueous solution; each milliliter contains 10 mg. of ritodrine hydrochloride, 0.44% (w/v) of acetic acid, 0.24% (w/v) of sodium hydroxide, 0.1% (w/v) of sodium metabisulfite, and 0.29% (w/v) of sodium chloride in water for injection USP. Hydrochloric acid or additional sodium hydroxide is used to adjust pH. Each Yutopar tablet contains 10 mg. of ritodrine hydrochloride.

Ritodrine hydrochloride is a white, odorless crystalline powder, freely soluble in water, with a melting point between 193° and 201°C. The chemical name of ritodrine hydrochloride is erythro-p-hydroxy-α-[1-[(p-hydroxyphenethyl) amino]ethyl] benzyl alcohol hydrochloride and has the chemical structure:



CLINICAL PHARMACOLOGY

Yutopar (ritodrine hydrochloride) is a beta-receptor agonist, which has been shown by *in vitro* and *in vivo* pharmacologic studies in animals to exert a preferential effect on the β_2 adrenergic receptors such as those in the uterine smooth muscle. Stimulation of the β_2 receptors inhibits contractility of the uterine smooth muscle.

In humans, intravenous infusions of 0.05 to 0.30 mg./min. or single oral doses of 10 to 20 mg. decreased the intensity and frequency of uterine contractions. These effects were antagonized by beta-blocking compounds. Intravenous administration induced an immediate dose-related elevation of heart rate with maximum mean increases between 19 and 40 beats per minute. Widening of the pulse pressure was also observed; the average increase in systolic blood pressure was 4.0 mm. Hg. and the average decrease in diastolic pressure was 12.3 mm. Hg. With oral intake, the increase in heart rate was mild and delayed.

During intravenous infusion in humans, transient elevations of blood glucose, insulin, and free fatty acids have been observed. Decreased serum potassium has also been found, but effects on other electrolytes have not been reported.

Serum kinetics in humans (non-pregnant females) of an intravenous infusion of 60 minutes duration were determined by measuring serum ritodrine levels by a radioimmunoassay technique. Three half-lives were calculated: the first of 6 to 9 minutes dominated the ascending phase of the serum drug-level curve; the second phase of 1.7 to 2.6 hours dominated the descending curve; and finally, a third phase, with a half-life of more than 10 hours, was discernible. In a study of serum kinetics after oral ingestion (male subjects), the decline of serum drug levels could be described in terms of a two-phase decay with an initial half-life of 1.3 hours and a final half-life of 12 hours. With either route of administration, 90% of the excretion was completed within 24 hours after the dose.

Comparison of ritodrine serum levels after intravenous administration with those after oral dosage indicates the oral bioavailability is about 30%. Intravenous infusion at a rate of 0.15 mg./min. for 1 hour yielded maximum serum levels ranging between 32 and 52 ng./ml. in a group of 6 non-pregnant female volunteers; maximum serum levels following single and repeated (4 x 10 mg./24 hr.) 10 mg. oral doses ranged between 5 and 15 ng./ml. and were obtained within 30 to 60 minutes after ingestion.

Placental transfer was confirmed by measurement of drug concentrations in cord blood showing that ritodrine and its conjugates reach the fetal circulation.

INDICATIONS AND USAGE

Yutopar is indicated for the management of preterm labor in suitable patients.

Administered intravenously, the drug will decrease uterine activity and thus prolong gestation in the majority of such patients. After intravenous Yutopar has arrested the acute episode, oral administration may help to avert relapse. Additional acute episodes may be treated by repeating the intravenous infusion. The incidence of neonatal mortality and respiratory distress syndrome increases when the normal gestation period is shortened.

Since successful inhibition of labor is more likely with early treatment, therapy with Yutopar should be instituted as soon as the diagnosis of preterm labor is established and contraindications ruled out in pregnancies of 20 or more weeks' gestation. The efficacy and safety of Yutopar in advanced labor, that is, when cervical dilatation is more than 4 cm. or effacement is more than 80%, have not been established.

CONTRAINDICATIONS

Yutopar is contraindicated before the 20th week of pregnancy.

Yutopar is also contraindicated in those conditions of the mother or fetus in which continuation of pregnancy is hazardous; specific contraindications include:

1. Antepartum hemorrhage which demands immediate delivery
2. Eclampsia and severe preeclampsia
3. Intrauterine fetal death
4. Chorioamnionitis
5. Maternal cardiac disease
6. Pulmonary hypertension
7. Maternal hyperthyroidism
8. Uncontrolled maternal diabetes mellitus (See Precautions.)
9. Pre-existing maternal medical conditions that would be seriously affected by the known pharmacologic properties of a betamimetic drug; such as: hypovolemia, cardiac arrhythmias associated with tachycardia or digitalis intoxication, uncontrolled hypertension, pheochromocytoma, bronchial asthma already treated by betamimetics and/or steroids
10. Known hypersensitivity to any component of the product

WARNINGS

Maternal pulmonary edema has been reported rarely in patients treated concomitantly with Yutopar and corticosteroids and one maternal death has been reported in such a patient. Patients so treated must be closely monitored in hospital. If pulmonary edema develops, the drugs should be discontinued and the edema managed by conventional means.

Intravenous administration of Yutopar should be supervised by persons having knowledge of the pharmacology of the drug and who are qualified to identify and manage complications of drug administration and pregnancy. Because cardiovascular responses are common and more pronounced during intravenous administration of Yutopar, cardiovascular effects, including maternal pulse rate and blood pressure and fetal heart rate, should be closely monitored. Care should be exercised for maternal signs and symptoms of pulmonary edema. Occult cardiac disease may be unmasked with the use of Yutopar.

The drug should not be administered to patients with mild to moderate preeclampsia, hypertension, or diabetes unless the attending physician considers that the benefits clearly outweigh the risks.

PRECAUTIONS

When Yutopar is used for the management of preterm labor in a patient with premature rupture of the membranes, the benefits of delaying delivery should be balanced against the potential risks of development of chorioamnionitis.

Among low birth weight infants, approximately 9% may be growth retarded for gestational age. Therefore, Intra-Uterine Growth Retardation (IUGR) should be considered in the differential diagnosis of preterm labor; this is especially important when the gestational age is in doubt. The decision to continue or reinitiate the administration of Yutopar will depend on an assessment of fetal maturity. In addition to clinical parameters, other studies, such as sonography or amniocentesis, may be helpful in establishing the state of fetal maturity if it is in doubt.

Laboratory Tests

Because intravenous administration of Yutopar has been shown to elevate plasma insulin and glucose and to decrease plasma potassium concentrations, monitoring of glucose and electrolyte levels is recommended during protracted infusions. Special attention should be paid to biochemical variables when treating diabetic patients or those receiving potassium-depleting diuretics.

Drug Interactions

Corticosteroids used concomitantly may lead to pulmonary edema. (See Warnings.)

The effects of other sympathomimetic amines may be potentiated when concurrently administered and these effects may be additive. A sufficient time interval should elapse prior to administration of another sympathomimetic drug.

Beta-adrenergic blocking drugs inhibit the action of Yutopar; coadministration of these drugs should, therefore, be avoided.

With anesthetics used in surgery, the possibility that hypotensive effects may be potentiated should be considered.

Carcinogenesis

In rats given oral doses of 1, 10, and 150 mg./kg./day of ritodrine hydrochloride for 82 weeks, no evidence of tumorigenicity was noted.

Pregnancy

Teratogenic Effects

(Pregnancy Category B)

Reproduction studies were performed in rats and rabbits. The doses employed intravenously were 1/9 (1 mg./kg.), 1/3 (3 mg./kg.), and 1 (9 mg./kg.) times the maximum human daily intravenous dose (but given to the animals as a bolus rather than by infusion). The oral doses, 10 and 100 mg./kg. represented 5 and 50 times the maximum human daily oral maintenance dose. The results of these studies have revealed no evidence of impaired fertility or harm to the fetus due to ritodrine hydrochloride.

No adverse fetal effects were encountered when single intravenous doses of 1, 3, and 9 mg./kg./day or oral doses of 10 and 100 mg./kg./day were given to rats and rabbits on Days 6 through 15 and 6 through 18 of gestation, respectively. Intravenous doses of 1 and 8 mg./kg./day or oral doses of 10 and 100 mg./kg./day administered to the mother from Day 15 of pregnancy to Day 21 postpartum did not affect perinatal or postnatal development in rats. A slight increase in fetal weight in the rat was observed. Oral administration to both sexes did not impair fertility or reproductive performance. Lethal doses to pregnant rats did not cause immediate fetal demise. There are no adequate and well-controlled studies of Yutopar effects in pregnant women before 20 weeks' gestation; therefore, this drug should not be used before the 20th week of pregnancy. Studies of Yutopar administered to pregnant women from the 20th week of gestation have not shown increased risk of fetal abnormalities. Follow-up of selected variables in a small number of children for up to 2 years has not revealed harmful effects on growth, developmental or functional maturation. Nonetheless, although clinical studies did not demonstrate a risk of permanent adverse fetal effects from Yutopar, the possibility cannot be excluded; therefore, Yutopar should be used only when clearly indicated.

Some studies indicate that infants born before 36 weeks' gestation make up less than 10% of all births but account for as many as 75% of perinatal deaths and one-half of all neurologically handicapped infants. There are data available indicating that infants born at any time prior to full term may manifest a higher incidence of neurologic or other handicaps than occurs in the total population of infants born at or after full term. In delaying or preventing preterm labor, the use of Yutopar should result in an overall increase in neonatal survival. Handicapped infants who might not have otherwise survived may survive.

ADVERSE REACTIONS

The unwanted effects of Yutopar are related to its betamimetic activity and usually are controlled by suitable dosage adjustment.

Effects Associated with Intravenous Administration

Usual effects (80-100% of patients)

Intravenous infusion of Yutopar leads almost invariably to dose-related alterations in maternal and fetal heart rates and in maternal blood pressure. During clinical studies in which the maximum infusion rate was limited to 0.35 mg./min. (one patient received 0.40 mg./min.), the maximum maternal and fetal heart rates averaged, respectively, 130 (range 60 to 180) and 164 (range 130 to 200) beats per minute. The maximum maternal systolic blood pressures averaged 128 mm. Hg (range 96 to 162 mm. Hg), an average increase of 12 mm. Hg from pretreatment levels. The minimum maternal diastolic blood pressures averaged 48 mm. Hg (range 0 to 76 mm. Hg), an average decrease of 23 mm. Hg from pretreatment levels. While the more severe effects were usually managed effectively by dosage adjustments, in less than 1% of patients, persistent maternal tachycardia or decreased diastolic blood pressure required withdrawal of the drug.

Yutopar infusion is associated with transient elevation of blood glucose and insulin, which decreases toward normal values after 48 to 72 hours despite continued infusion. Elevation of free fatty acids and cAMP has been reported. Reduction of potassium levels should be expected; other biochemical effects have not been reported.

Frequent effects (10-50% of patients)

Intravenous Yutopar, in about one-third of the patients, was associated with palpitation. Tremor, nausea, vomiting, headache, or erythema was observed in 10 to 15% of patients.

Occasional effects (5-10% of patients)

Nervousness, jitteriness, restlessness, emotional upset, or anxiety was reported in 5 to 6% of patients and malaise in similar numbers.

Infrequent effects (1-3% of patients)

Cardiac symptoms including chest pain or tightness (rarely associated with abnormalities of ECG) and arrhythmia were reported in 1 to 2% of patients.

Other infrequently reported maternal effects included: anaphylactic shock, rash, heart murmur, epigastric distress, ileus, bloating, constipation, diarrhea, dyspnea, hyperventilation, hemolytic icterus, glycosuria, lactic acidosis, sweating, chills, drowsiness, and weakness.

Neonatal Effects

Infrequently reported neonatal symptoms include hypoglycemia and ileus. In addition, hypocalcemia and hypotension have been reported in neonates whose mothers were treated with other betamimetic agents.

Effects Associated with Oral Administration

Frequent effects (<50% of patients)

Oral ritodrine in clinical studies was often associated with small increases in maternal heart rate, but little or no effect upon either maternal systolic or diastolic blood pressure or upon fetal heart rate was found.

Oral ritodrine in 10 to 15% of patients was associated with palpitation or tremor. Nausea and jitteriness were less frequent (5 to 8%), while rash was observed in some patients (3 to 4%), and arrhythmia was infrequent (about 1%).

OVERDOSAGE

The symptoms of overdosage are those of excessive beta-adrenergic stimulation including exaggeration of the known pharmacologic effects, the most prominent being tachycardia (maternal and fetal), palpitation, cardiac arrhythmia, hypotension, dyspnea, nervousness, tremor, nausea, and vomiting. If an excess of ritodrine tablets is ingested, gastric lavage or induction of emesis should be carried out followed by administration of activated charcoal. When symptoms of overdose occur as a result of intravenous administration, ritodrine should be discontinued; an appropriate beta-blocking agent may be used as an antidote. Ritodrine hydrochloride is dialyzable.

Acute intravenous toxicity was studied in rats and rabbits and acute oral toxicity in mice, rats, guinea pigs, and dogs. The LD50 values in the most sensitive of the species used were 64 mg./kg. intravenously in the nonpregnant rabbit and 540 mg./kg. orally in the nonpregnant mouse. The intravenous LD50 value in the pregnant rat was 85 mg./kg. The amount of drug required to produce symptoms of overdose in humans is individually variable. No reports of human mortality due to overdose have been received.

DOSAGE AND ADMINISTRATION

In the management of preterm labor, the initial intravenous treatment should usually be followed by oral administration. The optimum dose of Yutopar is determined by a clinical balance of uterine response and unwanted effects.

Intravenous Therapy

Do not use intravenous Yutopar if the solution is discolored or contains any precipitate or particulate matter.

Yutopar solution should be used promptly after preparation, but in no case after 48 hours of preparation.

Method of Administration: To minimize the risks of hypotension, the patient should be maintained in the left lateral position during infusion and careful attention given to her hydration.

For appropriate control and dose titration, a controlled infusion device is recommended to adjust the rate of flow in drops/minute. An i.v. microdrip chamber (60 drops/ml.) can provide a convenient range of infusion rates within the recommended dose range for Yutopar.

Recommended Dilution: 150 mg. ritodrine hydrochloride (3 ampuls) in 500 ml. fluid yielding a final concentration of 0.3 mg./ml. Ritodrine for intravenous infusion should be diluted with one of the following:

—0.9% w/v sodium chloride solution
—5% w/v dextrose solution
—10% w/v dextran 40 in 0.9% w/v sodium chloride solution
—10% w/v invert sugar solution
—Compound sodium chloride solution (Ringer's solution)
—Hartmann's solution

Intravenous therapy should be started as soon as possible after diagnosis. The usual initial dose is 0.1 mg./minute (0.33 ml./min., 20 drops/min. using a microdrip chamber at the recommended dilution), to be gradually increased according to the results by 0.05 mg./minute (0.17 ml./min., 10 drops/min. using a microdrip chamber at the recommended dilution) every 10 minutes until the desired result is attained. The effective dosage usually lies between 0.15 and 0.35 mg./minute (0.50 to 1.17 ml./min., 30-70 drops/min. using a microdrip chamber at the recommended dilution). The infusion should generally be continued for at least 12 hours after uterine contractions cease. With the recommended dilution, the maximum volume of fluid that might be administered after 12 hours at the highest dose (0.35 mg./min.) will be approximately 840 ml. The amount of i.v. fluids administered and the rate of administration should be monitored to avoid circulatory fluid overload. Frequent monitoring of maternal uterine contractions, heart rate, and blood pressure, and of fetal heart rate is required, with dosage individually titrated according to response.

Oral Maintenance

One tablet (10 mg.) may be given approximately 30 minutes before the termination of intravenous therapy. The usual dosage schedule for the first 24 hours of oral maintenance is 1 tablet (10 mg.) every two hours. Thereafter the usual dose is 1 or 2 tablets (10 to 20 mg.) every four to six hours, the dose depending on uterine activity and unwanted effects. The total daily dose of oral ritodrine should not exceed 120 mg. The treatment may be continued as long as the physician considers it desirable to prolong pregnancy.

Recurrence of unwanted preterm labor may be treated with repeated infusion of Yutopar.

HOW SUPPLIED

NDC 0068-0599-53:	5 ml. ampuls in boxes of 12. Each ampul contains 50 mg. (10 mg./ml.) of ritodrine hydrochloride.
NDC 0068-0595-60:	10 mg. tablets in bottles of 60. Each round, yellow tablet contains 10 mg. ritodrine hydrochloride and is inscribed YUTOPAR on one side and scored on the other.

Product Information as of July, 1980

Both the tablet and intravenous dosage forms should be stored at room temperature, preferably below 86°F. (30°C.). Protect from excessive heat.

Parenteral (intravenous) dosage form manufactured
by TAYLOR PHARMACAL COMPANY
Decatur, Illinois 62525
Distributed by
MERRELL-NATIONAL LABORATORIES
Division of Richardson-Merrell Inc.
Cincinnati, Ohio 45215, U.S.A.

Yutopar® licensed by
Duphar B.V.,
Amsterdam, Holland

Merrell

0-5690 (Y556B)

AUGUST, 1980

PRINTED IN U.S.A.

The use of human menopausal and chorionic gonadotropins for induction of ovulation

Sixteen years' experience at the Sloane Hospital for Women

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Gonadotropin therapy for anovulation is highly successful: 58.6% of treated patients conceive. Better results are achieved in patients with galactorrhea-amenorrhea (77.1%) and hypogonadotropic hypogonadism (63.3%) than in patients with normal gonadotropin levels (45.4%). The spontaneous abortion rate (27.5%) is somewhat higher than that in spontaneous pregnancies. The multiple pregnancy rate is 31% and was slightly lower in cycles with preovulatory estrogen levels in the physiologic range. In patients treated with human menopausal and chorionic gonadotropins for 7 to 9 days per cycle, the multiple pregnancy rate is considerably less (12.9%) than in patients with longer treatment. The efficacy of treatment does not diminish with repeat-treatment cycles.
(AM. J. OBSTET. GYNECOL. 138:801, 1980.)

THE POSSIBILITY of inducing ovulation with human pituitary gonadotropin was first demonstrated by Gemzell and associates.[1] Shortly thereafter the use of human menopausal gonadotropin (hMG) in combination with human chorionic gonadotropin (hCG) was introduced for the treatment of infertility resulting from disorders of the ovarian-hypothalamic-pituitary axis. This therapy has now been used for about 20 years with great success, particularly after methods for monitoring estrogen levels as a means of following the progression of the treatment were described by Ittrich[2] and Brown and Beisher.[3] Several reviews of hMG/hCG treatment have been published.[4-6]

In 1972, we reported on 101 patients who had been treated with hMG/hCG for 212 cycles.[7] The main objective of that study was to relate preovulatory estrogen levels to the incidence of successful pregnancies and complications such as ovarian hyperstimulation and multiple pregnancies.

The present paper represents a review of all treatment cycles given in this department to date, including those of the earlier years. The main consideration after

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Received for publication March 17, 1980.
Revised July 22, 1980.
Accepted July 31, 1980.
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0002-9378/80/230801+07$00.70/0 © 1980 The C. V. Mosby Co.

Table I. First conception and delivery of first living child

Group	*No. of patients treated*	*First conception*	*First successful pregnancy*
Group 1 (low gonadotropin levels)	109	69 (63.3%)	60 (55.0%)
Group 2 (normal gonadotropin levels)	88	40 (45.4%)	29 (32.9%)
Group 3 (elevated prolactin levels)	35	27 (77.1%)	26 (74.3%)
Total	232	136 (58.6%)	115 (49.6%)*

*Among these 115 patients were 12 who after further treatment had another successful delivery of a second child (or children). One woman had two single children followed by quintuplets at a third delivery, and one woman was delivered twice of single children and twice of twins.

Table II. Ovulations and conceptions in 655 hMG/hCG treatments given to 232 patients

Group	*No. of patients*	*Treatment cycles*	*Ovulatory cycles*	*Anovulatory cycles*	*Conceptions (% of ovulatory cycles)*
1	109	320	304 (95%)	16	90 (29.6%)
2	88	249	244 (98%)	5	48 (19.7%)
3	35	86	86 (100%)	0	44 (51.2%)
Total	232	655	634 (96.8%)	21	182 (28.7%)

this prolonged experience is to determine which groups of patients benefit most from this treatment and how long and which doses of hMG are optimal.

Material and methods

Patient selection and classification. From 1963 to 1979, 242 patients were treated during 674 cycles. Among them were 10 patients (13 cycles) with primary ovarian failure and high serum gonadotropin levels who did not respond as expected and were excluded from the following review. Included are nine patients with anovulation, with additional factors contributing to their infertility. Two had lysis of pelvic adhesions, two were treated for Asherman's syndrome, and five patients had husbands with low sperm counts.

All patients except those with galactorrhea-amenorrhea were grouped according to the classification suggested by Insler and associates[8] and by the World Health Organization. Group 1 comprised 89 patients who had no evidence of estrogen activity and low serum gonadotropin levels. This group contained patients with hypogonadotropic hypogonadism, including 15 with primary amenorrhea. The patients presented with a history of amenorrhea, an atrophic vagina, absent or minimal cervical mucus, and absence of withdrawal bleeding after progesterone administration. Baseline estrogen levels were lower than 10 μg/24 hours in the urine or lower than 80 pg/ml in the serum. Group 2 included 84 patients who had evidence of some endogenous estrogen activity and low or normal gonadotropin levels. These patients had a history of oligomenorrhea or amenorrhea for less than 6 months, demonstrated cervical mucus and an estrogenized vagina, and had withdrawal bleeding following progesterone administration. Baseline estrogen levels were higher than 10 μg/24 hours in the urine or greater than 80 pg/ml in the serum. When estrone and estradiol measurements were done separately, estrone was usually the major component. The majority of patients in this group were diagnosed as having the polycystic ovary syndrome.

Because of the importance of prolactin in the reproductive processes and the ability to measure it in the blood, patients with the galactorrhea-amenorrhea syndrome formed a third group. The diagnosis was based on clinical observation of galactorrhea-amenorrhea and, since 1974, prolactin levels were determined in all anovulatory patients. Group 3 included 35 women. If previous treatment for pituitary adenomas by radiation or operation was successful, those patients were included in Group 1 and only those patients with persistent galactorrhea or elevated prolactin levels were included in Group 3.

Mode of therapy. Pergonal was used as the hMG preparation in all patients. There is general agreement that the Pergonal administration schedule has to be adjusted individually. Since 1966, all of our patients have been started with two ampules of Pergonal daily (75 IU of follicle-stimulating hormone (FSH) and 75 IU of luteinizing hormone (LH) per ampule). The dose was then increased every 3 to 4 days as indicated by the ovarian response. The last dose of Pergonal was fol-

Table III. Outcome of conceptions

Group	*Conceptions*	*Abortions*	*Single pregnancies*	*Multiple pregnancies*	*Total No. of live births*
1	90	24 (26.7%)	42 (46.6%)	24 (26.7%)	66 (73.3%)
2	48	16 (33.3%)	25 (52.1%)	7 (14.6%)	32 (66.6%)
3	44	10 (22.7%)	24 (54.5%)	10 (22.7%)	34 (77.3%)
Total	182	50 (27.5%)	91 (50.0%)	41 (22.5%)	132 (72.5%)

Table IV. Multiple pregnancies

Group	*Twins*	*Triplets*	*Quadruplets*	*Quintuplets*	*Total*
1	18	5	1	0	24
2	5	1	1	0	7
3	5	4	0	1	10
Total	28	10	2	1	41

lowed by a single dose of 10,000 IU of hCG. The length of Pergonal therapy was usually 7 to 15 days but in individual cases it was extended up to 25 days. In patients treated before 1966, the treatment was more variable, particularly with respect to the dose of hCG.

Estrogen monitoring of ovarian response. In a small group of patients who received hMG/hCG before 1964, the degree of ovarian response was judged by clinical impression only. Starting in 1964, measurement of urinary estrogens was used to monitor the response. A preovulatory urinary estrogen level of 80 to 250 μg/24 hours was considered to be adequate.[7] Since 1977, serum estrogen levels determined by radioimmunoassay have been used. Preovulatory serum estrogen levels in the range of 500 to 1,500 pg/ml are considered optimal.

Evidence of ovulation. Occurrence of pregnancy provided definite proof of ovulation. Blood progesterone levels above 3 ng/ml (or urinary pregnanediol levels above 2 mg/24 hours) and/or biphasic temperature curves together with appropriately timed uterine bleeding provided indirect evidence of apparent ovulation.

Results

Among the 232 women treated for infertility, 136 conceived and 115 were delivered of living infants. The greatest percentage of successfully treated women was seen in Group 3 (74%); somewhat smaller success rates occurred in Group 1 (55%) and in Group 2 (33%). These data are shown in Table I.

Among the 115 patients with successful pregnancies, after further treatment, 12 had another successful delivery of a second child (or children). (See footnote to Table I).

The data on ovulation and conception for all treatment cycles are shown in Table II. Ovulation was induced in nearly all treatment cycles. The ovulation rate (ovulations per treatment cycle) was between 95% and 100% in all three groups of patients. The conception rate achieved during these cycles was 51.2% in Group 3 (the patients with galactorrhea-amenorrhea), 29.6% in Group 1, and 19.7% in Group 2. The conception rate was significantly higher ($P < 0.001$) in Group 3 than in Groups 1 and 2 and significantly lower ($P < 0.001$) in Group 2 than in Group 1. However, Group 2 excluded all women who had conceived following clomiphene administration. If this portion of our patient population had been treated with hMG/hCG instead of clomiphene, the conception rate in Group 2 might well have been higher.

Table III presents the outcome of the 182 conceptions. Successful pregnancies resulted in 132 patients (72.5%) while 50 pregnancies (27.5%) ended in abortion. Most of the abortions occurred in the first trimester and there were only a few in the second trimester. The abortion rate (as percent of conceptions) was slightly higher in Group 2 than in Groups 1 and 3. Among the successful pregnancies, 91 were single births and 41 were multiple births. The overall multiple-pregnancy rate was 31% of live births with a significantly lower percentage in Group 2 than in Groups 1 and 3 ($P < 0.001$). The breakdown of multiple pregnancies is shown in Table IV. There were 28 twins, 10 triplets, two quadruplets, and one set of quintuplets in our series.

Table V presents the number of treatment cycles administered before a patient achieved conception followed by a live birth. Of the 132 conceptions resulting in a living child 39.4% occurred after the first cycle. Women who desired repeat treatment after having a first child are included in this table as starting again with their first cycle. Up to 14 treatment cycles have been given to women who wished to continue treatment. However, a considerable number of patients chose to discontinue treatment at earlier stages, some even after the first or second cycle when these had not

Table V. Number of treatment cycles required to achieve conception and a live birth

Cycle	*No. of patients*	*Conceptions*	*Abortions*	*Successful pregnancies*	*Discontinued treatment without live birth*
1	242	74 (30.6%)	22 (9.1%)	52 (21.5%)	27
2	163	47 (28.8%)	10 (6.1%)	37 (22.7%)	39
3	87	23 (26.4%)	5 (5.7%)	18 (20.1%)	20
4	49	11 (22.4%)	3 (6.1%)	8 (16.3%)	9
5	32	10 (31.2%)	3 (9.4%)	7 (21.8%)	4
6	21	4 (19%)	2 (9.5%)	2 (9.5%)	5
7	12	5 (41.6%)	2 (16.6%)	3 (25%)	6
8	9	3 (33.3%)	1 (11.1%)	2 (22.2%)	1
9	6	3 (50%)	1 (16.7%)	2 (33.3%)	2
10	2	—	—	—	1
11	1	—	—	—	—
12	1	1	1	—	—
13	1	—	—	—	—
14	1	1	—	1	—
Total		182	50	132	114

Table VI. Days of Pergonal treatment required to attain sufficient ovarian stimulation

Days	*No. of ovulatory cycles*	*Conceptions*	*Abortions*	*Live births (% of conceptions)*	*Multiple pregnancies (% of live births)*
7-9	158	49 (31.0%)	18 (36.7%)	31 (63.3%)	4 (12.9%)
10-15	376	107 (28.4%)	21 (19.6%)	86 (80.4%)	34 (39.5%)
16-25	100	26 (26.0%)	11 (42.3%)	15 (57.7%)	3 (20.0%)

Table VII. Ampules of Pergonal given per ovulatory treatment cycle

	No. of treatment cycles		
No. of ampules	*Group 1*	*Group 2*	*Group 3*
9-19	42 (13.8%)	70 (28.7%)	6 (7.0%)
20-39	145 (47.6%)	133 (54.5%)	41 (47.7%)
40-59	61 (20.1%)	33 (13.5%)	24 (27.9%)
60+	56 (18.4%)	8 (3.3%)	15 (17.4%)

One ampule of Pergonal is equivalent to 75 IU of FSH plus 75 IU of LH.

resulted in pregnancy. These patients are listed in the last column of the table. The pregnancy rates suggest that the chances of conception and successful delivery do not decline significantly as the number of treatment cycles increases. Therefore, it appears justified to encourage patients to continue treatment, even beyond the first three cycles.

Not only the dose of Pergonal but also the time span over which it was administered appeared to influence the outcome of treatment. Pergonal was given over 7 to 25 days before ovulation was induced. In Table VI the duration of treatment is grouped according to short (7 to 9 days), intermediate (10 to 15 days), and prolonged (16 to 25 days) periods. The intermediate period reflects most closely the physiologic period of follicular maturation and it was this intermediate period which proved, in general, to be optimal. In this group the abortion rate was lowest, 19% as compared to 36% during the short and 42% during the prolonged treatment periods ($P < 0.05$). However, the incidence of multiple pregnancies was highest (39% versus 13% and 20%).

The amount of Pergonal administered to an individual patient per cycle is shown in Table VII. Satisfactory response was elicited with 9 to 19 ampules in a considerable number of patients in Groups 1 and 2, but rarely in Group 3. The most frequently used dose was between 20 and 39 ampules. Practically all patients in Group 2 responded to up to 59 ampules, whereas a considerable number of patients in Groups 1 and 3 needed more than 60 ampules of Pergonal.

Table VIII shows the extent of ovarian stimulation as judged by estrogen levels in blood or urine. The estrogen levels are grouped in such a way that the first group of the table represents levels corresponding to maximal midcycle physiologic levels, the second group represents levels found empirically to indicate ovarian stimulation optimal for induction of ovulation, and the third group represents extensive ovarian stimulation. While conception rates were similar in all three groups, the abortion rates were highest in the extensively stimulated group, a fact we reported earlier.[7] Consequently, the extensively stimulated group had 20% fewer suc-

Table VIII. Preovulatory estrogen levels and outcome of conceptions*

Estrogen levels	*No. of cycles*	*Conceptions (% of cycles)*	*Abortions (% of conceptions)*	*Live births (% of conceptions)*	*Multiple pregnancies (% of live births)*
<150 µg/24 hr (urine) <500 pg/ml (blood)	243	67 (27.6%)	16 (23.9%)	51 (76.1%)	13 (25.5%)
150-250 µg/24 hr (urine) 500-1,500 pg/ml (blood)	185	56 (30.3%)	16 (28.6%)	40 (71.4%)	13 (32.5%)
>250 µg/24 hr (urine) >1,500 pg/ml (blood)	159	49 (30.8%)	21 (42.8%)	28 (51.2%)	10 (35.7%)

*Blood levels on day of hCG; urine levels 1 to 2 days before hCG.

Table IX. Relationship between preovulatory estrogen levels and ovarian hyperstimulation

Estrogen levels	*Cycles without complication*	*Cycles with moderate hyperstimulation*	*Cycles with severe hyperstimulation*
<150 µg/24 hr (urine) <500 pg/ml (blood)	239 (98.4%)	4 (1.6%)	0
150-250 µg/24 hr (urine) 500-1,500 pg/ml (blood)	180 (97.3%)	5 (2.7%)	0
>250 µg/24 hr (urine) >1,500 pg/ml (blood)	123 (76.4%)	28 (17.9%)	8 (5%)
Total	542 (92.3%)	37 (6.3%)	8 (1.4%)

cessful pregnancies than the other two groups.

The multiple-pregnancy rate was somewhat lower in the patients with preovulatory estrogen levels in the physiologic range (25.5% versus 32.5% and 35.7%). Of the 13 multiple gestations in this group, 11 were twins, one was triplets, and one was quintuplets. In the group with moderately elevated preovulatory estrogen levels, there were five twins, six triplets, and two quadruplets, and in the group with markedly elevated preovulatory estrogens there were eight twins and two triplets.

In six of the 159 cycles in which excessive ovarian stimulation occurred, hCG was withheld in order not to induce ovulation. The incidence of hyperstimulation is analyzed in Table IX. In the majority of patients, no complications occurred, but in all treatment cycles 37 patients (6.3%) developed a moderate ovarian hyperstimulation syndrome, defined as ovarian enlargement of up to 10 cm. None required treatment. In eight cycles (1.4% of the treatments) severe hyperstimulation occurred, as defined by ovarian enlargement greater than 10 cm and/or ascites and hydrothorax, and the patients were hospitalized for observation and treatment. No severe hyperstimulation occurred in patients with preovulatory urinary estrogen levels of less than 250 µg/24 hours or serum estrogen levels of less than 1,500 pg/ml.

Among the 132 live births, there were two newborn infants with congenital anomalies—one with multiple congenital anomalies and another with Down's syndrome.

Comment

It is difficult to compare results of treatment with hMG-hCG among different investigators because of major differences in patient selection and the number of Pergonal treatment cycles given to an individual patient. Thompson and Hansen[4] summarized results from 100 investigators and found that 20% to 28% of patients conceived. Gemzell[6] reported that 44.3% conceived, and Spadoni and associates[9] reported a 35.5% conception rate. A total of 58.6% of our patients conceived.

There is also a marked difference in conception rates between groups of patients. The conception rate in Group 1 (63.3%) was higher than in Group 2 (45.4%), a finding consistent with results reported by Oelsner and colleagues.[5] The main difference between Group 1 and Group 2 is that patients in Group 2 had been preselected: all of them had experienced failure of clomiphene. The combined conception rate in Group 2 would have been considerably higher if both clomiphene- and Pergonal-treated patients were included. Ellis[10] noted that the higher the basal estrogen levels, the less successful the hMG treatment. High basal estrogen levels may be due to intraovarian pathology resulting from high local levels of androgens or to the presence of many follicles at various stages of maturation prior to treatment, some of which will be exhausted by the treatment while the others will be understimulated.

The highest conception rate (77.1%) occurred in the amenorrhea-galactorrhea group. Thompson and Hansen[4] also noted better results in this group. The high prolactin level did not seem to increase the abortion rate by causing corpus luteum defects as might be expected. A significant observation was that the length of hMG treatment during the cycle had a significant bearing on the outcome of pregnancy. In patients given Pergonal for less than 9 days, the abortion rate was higher than in patients treated for a longer period of time. A possible explanation is that the follicle was immature, resulting in an abnormal corpus luteum. The multiple-pregnancy rate was significantly lower (12.9%) in this group, perhaps because fewer follicles matured.

The effectiveness of treatment does not diminish with repeated treatment cycles and the conception rate is about the same in the fifth treatment cycle as in the first. Therefore one may advise patients to continue therapy until pregnancy occurs if no other infertility problems are detected.

The abortion rate in our patients was 27%, which is comparable to the 30% reported by Gemzell.[6] The abortion rate in spontaneously occurring pregnancies is under debate, making comparisons difficult. The reasons for the higher abortion rate reported in Pergonal-treated patients include the higher multiple-pregnancy rate and early diagnosis of pregnancy leading to recognition of very early abortion, which otherwise would have been missed. The data show that the abortion rate in Group 2 tended to be higher than in the other groups. In the patients with high preovulatory estrogen levels the abortion rate was significantly higher than in those with lower estrogen levels. A possible explanation is the formation of multiple corpora lutea that later degenerate, resulting in a marked decrease in the progesterone level in early pregnancy.

Our multiple pregnancy rate was 31%, almost the same as the 32.5% rate cited by Oelsner and coworkers.[5] There is a debate in the literature about the correlation between preovulatory estrogen levels and the multiple-pregnancy rate. Some authors[11-13] have found a relationship and they claim that lower preovulatory estrogen levels will reduce the rate of multiple gestations; however, the number of patients they have presented is small. In a previous publication[7] and in agreement with others[3, 14] we did not find a correlation. Our present data show only a slightly lower rate of multiple gestation in the group with preovulatory estrogen levels in the physiologic range. Eleven of the 13 multiple gestations in this group were twins.

Since our first report of hyperstimulation syndrome in one of our patients,[15] we have had seven additional patients with the syndrome who required hospitalization. The severe hyperstimulation rate was 1.4% of the total number of treatment cycles. In a review of the literature, Schenker and Weinstein[16] quoted the frequency of severe hyperstimulation as 0.8% to 2%. Moderate hyperstimulation occurred in 6.3% of the treatment cycles. All of our patients recovered completely. In the majority of patients with extensive ovarian stimulation no complication occurred. Ovarian hyperstimulation can be prevented by withholding hCG administration in patients with high preovulatory estrogen levels.

In conclusion, treatment with hMG-hCG for induction of ovulation is highly successful, and the complication rate is low. The recommended duration of treatment is 9 to 10 days per treatment cycle. Treatment can be repeated if necessary in patients without other infertility problems.

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Three metronidazole-resistant strains of *Trichomonas vaginalis* from the United States

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Trichomonas vaginalis isolates were obtained in the United States from three women with trichomoniasis refractory to routine treatment with metronidazole. Cure was obtained in these cases with increased dosage of metronidazole. In experimental mouse infections, all three strains showed a significantly (about 10 to 20 times) lower susceptibility to metronidazole than did a number of control strains. In vitro, the lowered susceptibility could be detected only in an aerobic assay but not in the routine anaerobic assay. The conclusion is that *T. vaginalis* strains with lowered metronidazole susceptibility do exist. Such strains might be responsible for a certain, but as yet entirely unknown, number of failures in routine metronidazole therapy of human trichomoniasis. (AM. J. OBSTET. GYNECOL. 138:808, 1980.)

HUMAN trichomoniasis is treated almost exclusively with metronidazole[1-3] and other 5-nitroimidazole derivatives, such as tinidazole, ornidazole, secnidazole, and nimorazole.[1, 3] Single courses of treatment have a failure rate of about 10%. This relatively low rate, however, represents a significant number of patients because of the prevalence of *Trichomonas vaginalis* infections. Reinfection and failure to take the medication are common causes of persistence or recurrence of trichomoniasis. Although no reliable data are available in a certain number of cases, even several courses of appropriate treatment fail to eradicate the infection. The total amount of metronidazole taken by such patients is often considerable. Several causes for such therapeutic failures are usually mentioned: (a) problems of a pharmacokinetic nature, i.e., insufficient absorption or transport of the drug to the site of infection,[4, 5] (b) the presence of a vaginal microflora that contains bacteria which inactivate the drug,[5-7] and (c) resistance of the infecting *T. vaginalis* to metronidazole.[8]

The last possibility was usually dismissed because concentrations of metronidazole that are easily reached during normal chemotherapy were sufficient to inhibit or kill, in in vitro tests, most trichomonad strains isolated from persistent infections. However, recent work demonstrated that most in vitro assays are not suitable to detect nitroimidazole resistance in *T. vaginalis*[9] and in other trichomonad species.[10] Thus, the lack of documented resistant isolates could be explained either by their genuine absence in human infections or by the unsuitability of the assay used. On the basis of the work with cattle trichomonads, a modified, aerobic in vitro assay was developed which seemed to overcome this difficulty.[10]

An isolate (strain IR-78) obtained in Vienna, Austria, from a patient with metronidazole-refractive trichomoniasis[11] was tested with this aerobic assay and was found to have a significantly lowered in vitro metronidazole susceptibility.[9] The lowered susceptibility was also detected in an in vivo assay in mice. Another resistant strain was isolated also in Gothenburg, Sweden.[12] These findings reopened the question of resistance to metronidazole as a potential therapeutic problem in human trichomoniasis.

In this report, we wish to draw attention to this ques-

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Received for publication January 14, 1980.

Revised June 23, 1980.

Accepted July 21, 1980.

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**Recipient of United States Public Health Service Grants AI 11942 and AI 12932 from the National Institute of Allergy and Infectious Diseases.*

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Table I. Metronidazole susceptibility of *Trichomonas vaginalis* isolates

		In vivo test			*In vitro test*	
		CD_{50}*	CD_{95}†		*Aerobic*	*Anaerobic*
Isolate	*Origin*	*mg/kg (3 ×)*		*Number of mice tested*	MLC_{24}‡ *(μg/ml)*	
Boston	Case 1	61	103	100	75	5
Albany	Case 2	60	81	60	60	4
Fall River	Case 3	82	150	105	250	2
IR-78	Vienna[7]	>800	>>800	Data from Reference 7	150	4
30001	ATCC	Not infective to mice			2	0.5
NYH209	Random isolates from New York Hospital	4.4	9.0	33	25	1
NYH272		3.1	6.2	36	6	1
NYH286		7.6	8.5	36	10	0.5
A	Vienna[7]	8.0	15	109	5	1

*Dose needed to cure 50% of the infections.
†Dose needed to cure 95% of the infections.
‡Minimum lethal concentration.

tion by presenting findings that indicate that similar *T. vaginalis* strains do occur in the United States. During 1978 and 1979, one of us (M. M.) received three inquiries concerning therapeutic failures in trichomoniasis. Isolates were obtained from the three patients by their attending physicians. With appropriate tests, all three isolates showed significantly lower metronidazole susceptibility than did control isolates. The results are compatible with the belief that treatment failures in human trichomoniasis can be due to relative drug resistance of the infecting *T. vaginalis* and are not always caused by host factors independent of the pathogen. Certain results of this study were presented at the Thirty-third Annual Meeting of the Society of Protozoologists (Washington, D. C., June, 1980). The physiologic and biologic properties of the isolates will be described elsewhere.

Case histories

Since the aim of the present study was to evaluate metronidazole susceptibility of three *T. vaginalis* strains, the case histories are presented here only as clinical background. None of the cases discussed responded to repeated standard metronidazole treatment (250 mg oral tablets three times a day for 7 days). On the basis of information in Reference 11, the individual physicians who treated these patients designed treatment schedules by increasing the amount of metronidazole administered in combination with other drugs. Although these schedules led to the elimination of the pathogen, they were not evaluated in detail, and we emphasize that they do not represent schedules recommended by us. Such schedules will have to be developed in the future.

Case 1. This 24-year-old, gravida 0, white woman was first seen with a 10-month history of recurrent vaginal infections caused by *T. vaginalis*. Both she and her only male consort had been given seven separate courses of metronidazole therapy, without relief of symptoms. Since initial examination by one of us (W. A. M.) disclosed that she had marked trichomonas vaginitis, she was again treated with a standard course of metronidazole. After therapy, microscopic examination of a wet mount of vaginal secretion still revealed many trichomonads. Because of epilepsy, this patient was taking phenobarbital and Dilantin. Analysis showed levels of phenobarbital well above the therapeutic range. On the hope that the excessive levels of phenobarbital in the blood had interfered with hepatic metabolism of metronidazole, the phenobarbital dosage was decreased and she was given a 14-day course of oral metronidazole in standard dosage with the concomitant use of Tricofuron vaginal suppositories. Although she was asymptomatic for 2 months, symptoms returned again and a wet mount examination showed many trichomonads. Cultures of *T. vaginalis* were obtained from the vaginal secretions at this time for testing of metronidazole susceptibility (Table I). The patient was given oral metronidazole in standard dosage supplemented with metronidazole vaginal tablets twice a day for 14 days. Unconjugated estrogen vaginal cream, three times a week, was also given during this period. Her partner received the same course of oral metronidazole. Although there was a symptomatic improvement for 2 months, the patient again became symptomatic, with trichomonads detectable in wet mount examination. For symptomatic relief, a regimen of oral tetracycline, 500 mg four times a day, and Betadine douches, twice a day, was used for 1 month. This provided no relief. To achieve higher serum and tissue levels of metronidazole, a treatment course of oral metronidazole, 2.5 gm three times a day for 3 days, was given to the patient and her male sexual partner. This resulted in the relief of symptoms, and, for the first time, the wet mount preparation of vaginal secretions was free of trichomonads. At the time of preparation of this report, more than a year after the last treatment, the patient is still free of symptoms.

Case 2. This 40-year-old, gravida 3 para 3, white woman was evaluated for recurrent *T. vaginalis* vaginitis. She had been treated three times with metronidazole and once with a local sulfonamide cream, without success. Her past history included a total hysterectomy for menorrhagia, in 1972. *T. vaginalis* was cultured from vaginal secretions, and its metronidazole susceptibility was determined (Table I). She was given two separate treatment courses of oral tetracycline, 250 mg four times a day for 3 weeks, and Premarin, 1.25 mg orally daily for 3 weeks, followed by a standard course of metronidazole. Her husband also received metronidazole therapy. The wet mount examination of vaginal secretions remained positive for trichomonads. Subsequently, she was given metronidazole tablets, 250 mg, to be inserted into the vagina twice a day for 3 days, but the wet smear of vaginal secretions remained positive. The final treatment regimen was oral metronidazole tablets, 500 mg three times a day for 7 days, for both her and her husband, plus the vaginal use of Aci-Jel, twice a day. The patient became asymptomatic and has remained so up until the time of preparation of this report.

Case 3. This patient was evaluated for a recurrent *T. vaginalis* vaginitis. A sample of the vaginal secretions containing many motile trichomonads was cultured, and the susceptibility of the isolated organisms was tested (Table I). She was treated with oral metronidazole, 1.0 gm twice a day for 10 days, as well as with a conjugated estrogen tablet, 1.25 mg daily for the 10 days. The patient experienced amelioration of symptoms on this treatment, and on follow-up examination, no trichomonads were seen in the wet mount and none was grown on culture.

Microbiologic methods

Secretion was collected from the vaginal fornix on cotton swabs which were subsequently placed in test tubes containing 7 or 8 ml of Diamond's TYM medium[13] (with agar), pH 6.0, and supplemented with 10% heat-inactivated horse serum and with 250 U/ml penicillin G and 250 μg/ml of streptomycin sulfate to suppress bacterial growth. The tubes were incubated at 37° C. In two cases, 50 μg/ml of gentamicin had to be used in subsequent transfers to eliminate bacterial contamination. Bacteria-free cultures were obtained by the third to fifth subculture. The isolates were passaged every other day for a few months and thereafter every day into the same medium without antibiotics. For future reference, the strains were preserved in liquid N_2.

T. vaginalis strains of known in vitro susceptibility to metronidazole were included in the study. These were the laboratory strain ATCC 30001, the resistant IR-78, and the susceptible A strains from Vienna,[9] as well as fresh strains (NYH 209, 272, 286) isolated from patients at New York Hospital (New York, New York).

Metronidazole susceptibility of the isolated organisms was tested as described earlier.[9, 10] The in vitro assays were done in New York (M. M.) and the in vivo assays in Vienna (J. G. M.). The in vivo sensitivity of the isolates was determined in mice injected with 10^6 organisms into each flank subcutaneously. Although organisms were inoculated also intraperitoneally into the same mice, infection was not obtained consistently, and the results are not presented. Metronidazole was administered in various quantities per os three times (2, 18, and 24 hours) after inoculation. The presence or absence of trichomonads at the site of injection was ascertained by necropsy on the sixth day after infection. The doses needed to cure 50% (CD_{50}) or 95% (CD_{95}) of the infections were calculated.[9] The in vitro assays were performed in plastic MultiWell tissue culture plates with 24 wells each (No. 3008, Falcon, Oxnard, California). Into each well was placed 1.4 ml of fluid thioglycollate medium, United States Pharmacopeia (Baltimore Biological Laboratory, Cockeysville, Maryland) adjusted to pH 6.0 and supplemented with 10% heat-inactivated horse serum and antibiotics (penicillin G, 400 U/ml, and streptomycin sulfate, 400 μg/ml). Aqueous metronidazole solutions in concentrations that ranged from 15 μg/ml to 3 mg/ml were added to the wells in order to obtain a series of final concentrations in the range desired. The volume of metronidazole solution added to each well was from 10 to 75 μl. Finally, 50 to 100 μl of a suspension containing 10^5 *T. vaginalis* cells was added to each well. Thus, the final volume of liquid in the wells varied by less than 10%, for which no compensation was made. The plate was covered with its own lid, which permitted equilibration with the surrounding gas phase. In the anaerobic assay the plates were placed into an anaerobic jar (GasPak Anaerobic System, Baltimore Biological Laboratory, Cockeysville, Maryland), whereas in the aerobic assay they were placed free into the incubator. After 24 hours of incubation at 37° C, the plates were examined with an inverted phase contrast microscope for the presence of motile cells. The lowest dilution in which no motile cells were seen was taken as the minimum lethal concentration (MLC). All values reported represent the means of at least three separate assays.

Microbiologic results

Subcutaneous infections elicited in mice by the Boston, Albany, and Fall River strains could be cured with metronidazole, but only if it was given at higher doses than necessary to eliminate infections with the control strains (Table I). The CD_{95} of these isolates was in the range of 3× 80 to 3× 150 mg/kg, whereas the values for the three control strains from New York were in the range of 3× 6 to 3× 9 mg/kg. The latter data compare well with CD_{95} values for the Vienna control strain A and with CD_{95} values of <3× 35 mg/kg published for 70 random isolates from Vienna.[10] The CD_{50} values were also significantly higher for the three isolates than for the controls. On the basis of these data, the Boston, Albany, and Fall River strains of *T. vaginalis* should be regarded as relatively resistant to metronidazole in mouse infections. However, in no isolate from the United States did the level of resistance reach that seen in strain IR-78.

MLC values observed in the aerobic in vitro test for these strains reflected the in vivo differences (Table I). The three fresh resistant isolates and the IR-78 strain were eliminated by 60 to >100 μg/ml of metronidazole. MLC values for the control strains were in the range of 6 to 25 μg/ml. Although some strain differences were found in the anaerobic MLC values, they all were in the range which is regarded as reflecting normal susceptibility.

At the time of the revision of this report (June, 1980), the IR-78 strain had been in culture without drug pressure for over 2 years, and the Boston strain for a year and a half. Periodic examination of their in vivo and in vitro susceptibility did not reveal any changes, thus suggesting that the resistance is a rather stable property.

Comment

Metronidazole was introduced about 20 years ago as an excellent drug for the treatment of human trichomoniasis, and it is frequently prescribed in the United States and in other countries.[1-3] Repeated warnings have been published that frequent use of it might lead to the development of drug-resistant strains of *T. vaginalis*.[1, 8] Although several investigators succeeded in developing in laboratory experiments strains of *T. vaginalis* and other trichomonads with markedly decreased susceptibility to this drug,[3, 8] the role of metronidazole resistance as a cause of treatment failures in cases of human trichomoniasis remained an open question.

In retrospect, it seems that one of the most important reasons for this situation rested on the available assay systems. *T. vaginalis* strains were often isolated from cases in which metronidazole was ineffective, and the strains were tested for drug sensitivity in vitro. Although some differences were observed in drug sensitivity, the inhibitory drug concentrations reported were usually in the range of 0.25 to 16 μg/ml,[1, 3, 5, 6, 8] levels which are easily reached in the blood and tissue fluids during standard metronidazole therapy.[14] Since such results suggested that the organisms should have been eliminated by the treatment, other factors were invoked to explain the therapeutic failures.[4-7] Testing of infections obtained in experimental animals was rarely done, although tests in animals could reflect the in vivo situation better.

In 1978, Thurner and Meingassner[11] studied a case of treatment failure of recurrent trichomoniasis in Austria. The isolate obtained showed in a mouse assay significantly lower susceptibility to metronidazole, tinidazole, and ornidazole than random isolates.[9] Their results strongly suggested resistance to the drug as the primary cause of the treatment failure. Isolates from the cases discussed in the present report showed properties similar to those of the Vienna isolate. The results demonstrate the existence in the United States of *T. vaginalis* strains resistant to metronidazole; thus the two strains isolated in Europe[11, 12] do not represent unique occurrences.

Using the modified aerobic assay,[9] we were able to detect metronidazole resistance in vitro in these isolates. It is noteworthy that, if the assays were performed under anaerobic conditions, i.e., similarly to the routine assays, the strains were more susceptible to metronidazole and were killed by drug concentrations that are regarded as easily reached in the blood and tissues.

The three isolates showed, in mice, tenfold to twentyfold lower susceptibility than random isolates. This finding suggests that resistance was the primary factor in these three treatment failures. The usual treatment schedule, i.e., 250 mg three times a day for 7 days, might not have resulted in sustained levels in the blood high enough to eliminate these organisms.[14] That a cure was obtained in all cases with increased metronidazole dosage supports this conclusion.

Further studies are needed to clarify the possible additional role of other factors and especially to establish the correlation between the resistance determined in vitro and the drug dosage necessary to eliminate the infection. Since there is concern about the long-term safety of metronidazole treatment,[1-3] the aim is to use the minimum dosage necessary. Recognition of the existence of the problem of resistance and the design of appropriate treatment schedules might, however, eliminate the exposure of certain patients to several repeated courses of standard therapy.

The frequency of occurrence and, thus, the overall significance of nitroimidazole resistance in *T. vaginalis* cannot yet be assessed. Although resistance should be considered in case of treatment failures, it is not likely to be their most frequent cause. This is suggested by the consistently high cure rate with metronidazole treatment, which has not changed significantly since the introduction of the drug. This conclusion is supported by the Vienna survey in which no resistance was detected among 94 random isolates.[15] Answers to these questions will be obtained only through an extensive survey of a random population, in which in vitro findings on susceptibility will be correlated with clinical results, and through a careful study of treatment failures.

The collaboration of Dr. A. Ellman (Albany, New York) and Dr. A. O. Anctil (Fall River, Massachusetts) in providing specimens and clinical data is acknowledged. We also wish to thank Mrs. E. T. Wolff, Mr. L. Wassermann, and Ms. R. Carroll for their excellent assistance. The ATCC 30001 strain of *T. vaginalis* was obtained from the American Type Culture Collection (Bethesda, Maryland), and the metronidazole used at The Rockefeller University was provided by G. D. Searle & Co. (San Juan, Puerto Rico) through the courtesy of Ms. E. Roman.

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Site of recurrence of cervical cancer after radical hysterectomy

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Of 564 patients who underwent radical hysterectomies for cervical cancer, 104 had recurrences. Twenty (3.5%) had recurrence in the central pelvis, and in nine (1.6%), this was the only site of recurrence. Of the patients who had lymph node metastases at the time of radical hysterectomy, 40% subsequently had recurrence compared with 14% who had negative nodes and recurrence. The site of recurrence, however, had no relationship to lymph node involvement, size of the lesion, stage of disease, cell type or grade, or previous pelvic irradiation. (AM. J. OBSTET. GYNECOL. 138:813, 1980.)

MOST REPORTS on the treatment of cervical cancer, by either radical surgery or irradiation, tend to concentrate on survival and complications of treatment as a means of assessing the effectiveness of treatment. A factor often overlooked is the site of recurrence. A recurrence in the central pelvis, whether after radical hysterectomy or pelvic irradiation, and especially if there is no spread of disease beyond the treated area, must be regarded as a reliable indicator of the effectiveness of treatment.

Material

The series involved 564 patients with cervical cancer treated by radical hysterectomy and pelvic lymphadenectomy at the Mayo Clinic from 1956 through 1975. Cancer of the cervix accounted for 92.5% of all radical hysterectomies performed during this period (Table I). Most of the patients had Stage IB (53.9%) and Stage IIA (21.3%) lesions (Table II). Of the 564 patients, 141 had previous irradiation as their primary treatment, and radical hysterectomy was being performed as a "salvage" procedure for radioresistant or recurrent disease. Of the 564 patients, 81.7% had squamous cell cancers and 15.1% had adenocarcinomas.[1]

Results

Of the 564 patients, 104 (18.4%) had recurrent cervical cancer (Table III). Central pelvic recurrence developed in 20 instances (3.5%), but in only nine instances (1.6%) was the central pelvis the only site of recurrence.

An initial analysis was performed on each of four major subgroups to endeavor to determine if any particular characteristic of the tumor predisposed the patient to recurrent disease in each of the four major sites: central pelvis, pelvic sidewall, abdominal cavity, and other sites (lung, bone, distant nodes, and so forth) (Table IV).

Size of lesion. The size of the cervical tumor was not always recorded by the pathologist, but when this information was available, tumors were placed into two groups according to greatest diameter. Of the 17 patients with central recurrences, 11 (65%) had tumors of 4 cm or less, but overall the groups were fairly evenly distributed, and statistical analysis showed no significant difference in relation to size of the lesion.

Stage of disease. Because many of these patients had had radiotherapy as their primary treatment at other institutions and were referred to our clinic with recurrent disease, the initial clinical stage was available for only 88 of the 104 patients. No significance was attributed to initial stage of disease in relation to site of recurrence.

Previous radiation therapy. Of the 104 patients with recurrent disease, 36 had had previous pelvic irradiation, and because these patients already had recurrent central pelvic tumor (that being the reason they un-

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Received for publication May 2, 1980.

Accepted July 30, 1980.

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0002-9378/80/230813+05$00.50/0 © 1980 The C. V. Mosby Co.

Table I. Distribution of patients according to sites of origin of cancer

Site	Patients No.	Patients %
Cervix	564	92.5
Endometrium	27*	4.4
Vagina	15*	2.4
Vulva	2	0.3
Colon	1	0.2
Breast	1	0.2

*One patient also had squamous cell carcinoma of the vulva.

Table II. Distribution of patients according to stage of disease in cancer of the cervix

Stage	Patients No.	Patients %
IA	59	10.5
IB	304	53.9
IIA	120	21.3
IIB	21	3.7
IIIA	9*	1.6
IIIB	1*	0.2
IV	3*	0.5
Unknown	47*	8.3
Total	564	100.0

*All had previously received radiotherapy.

derwent radical hysterectomy), a higher percentage of previously irradiated patients in the central recurrence group (55%) is expected when compared with the overall incidence (35%). However, statistical analysis did not reveal any significant relationship between site of recurrence and previous radiation therapy. One might expect fewer pelvic recurrences (central and sidewall) in the previously irradiated group owing to the sterilizing effect of the radiation treatments within the treated field. This was not so, as 38% of the patients with pelvic recurrences had had previous irradiation, the explanation being that operation was performed because radiotherapy had failed to control the disease.

Cell type. No significant difference in sites of recurrence could be found when squamous cell carcinoma was compared with adenocarcinoma of the cervix. Adenocarcinomas accounted for 10% of the central pelvic recurrences and 15% of all pelvic (central and sidewall) disease, being similar to the overall incidence of adenocarcinoma in the series (15%).

Table III. Distribution of patients according to sites of recurrence

Site	Patients	
Central:	20	
Pelvis		9
Plus distant		2
Plus sidewall		9
Sidewall:	41	
Plus distant		10
Alone		31
Abdominal:	18	
Cavity		13
Cavity and other		5
Other:	25	
Lung		9
Lung and other		5
Nodes		8
Vulva and perineum		3
Total	104	

Histologic grade. In the whole series of 564 cancers of the cervix, 80% had high-grade tumors (Broders grades 3 and 4), and there were 85% high-grade tumors in the group of 104 recurrences. However, there was no significant difference when site of recurrence was compared with grade of tumor.

Lymph node involvement. No significant differences were found between positive nodes and site of recurrence. However, when the 104 patients with recurrent disease were compared with the 460 patients who did not develop recurrence, 40% of those with positive nodes subsequently developed recurrence, compared with only 14% (65 patients) of those patients with noninvolved nodes. This was a highly significant difference ($P < 0.0001$) (Table V). Interestingly, the patients in whom pelvic sidewall recurrence developed had the highest incidence of positive nodes at hysterectomy (41.5%). This may be due to the failure to remove all involved lymphatic tissue at operation, thus predisposing the patient to recurrence in this region.

Reassessment

Because no relationships were found when recurrences were grouped into the previously mentioned four categories, data were reassessed by dividing the recurrences into three groups: (1) central pelvic disease only, (2) regional disease (disease involving the central pelvis and sidewall or sidewall alone but not outside the pelvis), and (3) distant disease (disease that may or may not involve the pelvis but is outside the pelvis) (Table VI).

Size of lesion. Large cervical lesions did not predispose to central recurrence, and the ratios were evenly distributed in the "regional" and "distant" categories.

Stage of disease. No significant differences could be found among the various categories.

Previous radiation therapy. Although most recurrences in the total group were noted in the patients not previously irradiated (and this applied also to the "regional" and "distant" groups), "central" recurrences

Table IV. Comparison of factors and sites of recurrence in patients with cervical cancer who had undergone radical hysterectomy

	Site of recurrence				
	Central	*Abdominal*	*Sidewall*	*Other*	*Total*
Lesion size (cm):					
≤4	11	2	14	8	35
>4	6	6	15	6	33
Total	17	8	29	14	68
Lesion Stage:					
I	6	9	13	11	39
II	8	7	18	11	44
III	2	0	1	1	4
IV	0	0	1	0	1
Total	16	16	33	23	88
Previous treatment:					
Radiation	11	7	12	6	36
No radiation	9	11	29	19	68
Total	20	18	41	25	104
Cell type:					
Squamous	17	17	34	18	86
Adenocarcinoma	2	1	7	5	15
Other	1	0	0	2	3
Total	20	18	41	25	104
Lesion grade:					
1 and 2	3	3	7	3	16
3	12	11	25	13	61
4	5	4	9	9	27
Total	20	18	41	25	104
Lymph nodes:					
Positive	5	7	17	10	39
Negative	15	11	24	15	65
Total	20	18	41	25	104

were evenly distributed. This is also probably influenced by this highly selected group who had failure of radiotherapy yet had a surgically removable, centrally recurrent tumor.

Patients who had not had irradiation before radical hysterectomy were also analyzed independently, with the use of all of the above criteria. Again, no significant differences were found.

Cell type. No significant differences in recurrence were noted between cell types.

Histologic grade. Histologic grade did not influence the site of recurrence.

Lymph node involvement. Only one patient in the group with purely central pelvic recurrences (11%) had lymph node metastasis at lymphadenectomy, and this patient had only one metastatically involved node. A higher percentage of patients in the "regional" (35%) and "distant" (43%) groups had node metastasis at operation, but there was no statistical difference between the groups.

Percentage vaginectomy. The percentage vaginectomy was assessed and was used as a measure of how radical the procedure was. Central recurrences were more frequent when more than 25% of the vagina was excised; however, this difference was not statistically significant.

Survival. Only six (5.7%) of the 104 patients have survived their recurrent disease. Although this percentage appears to be very low, only nine of the 104 had recurrent disease that was confined to the central pelvis, and these nine are the only patients who were potentially salvageable by pelvic exenteration. However, all nine patients died of disease. Calame,[2] in reviewing 193 recurrent cervical cancers, noted a survival rate of 4.1%. The steep slopes of the survival curve reflect the short survival of the patients in each group once there was recurrence (Fig. 1).

Table V. Relation of recurrence to nodal involvement in patients with cervical cancer who had undergone radical hysterectomy

	Recurrence		
Lymph nodes	*Yes*	*No*	*Total*
Positive	39	58	97
Negative	65	402	467
Total	104	460	564

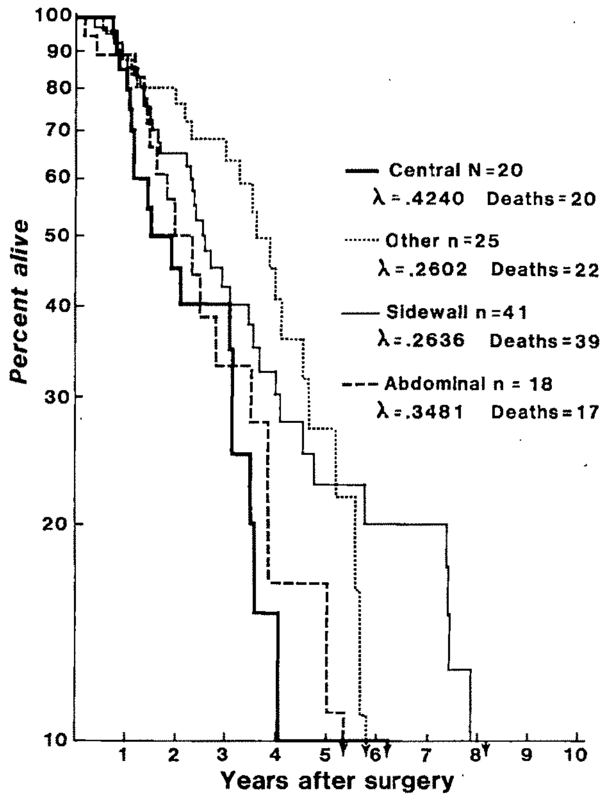

Fig. 1. Survival of patients according to site of recurrence of cervical cancer after radical hysterectomy.

Comment

Central pelvic recurrences must be the main reference point for judging the adequacy of both operation and radiotherapy for cervical cancer. Obviously, if the cervical tumor cannot be excised with wide margins of normal tissue, then local recurrence is likely. Unfortunately, with cervical cancer, the close approximation of the ureters, bladder, and rectum requires compromising this surgical principle, and avoiding these structures may result in an inadequate radical procedure. A central recurrence rate of only 1.6% in this series of 564 radical hysterectomies shows that the degree of radicality of the procedure which we are performing is satisfactory. Even when one includes the other 11 patients who had central disease as well as disease elsewhere (nine within the pelvis and two distant), the central recurrence rate is 3.5%. However, recurrence that develops simultaneously at other sites along with the central lesion probably is a measure of the biological behavior of the tumor rather than the adequacy of surgical resection.

Even when irradiation is used as the primary method of treatment, central recurrence has been a problem. Cullhed[3] reported a recurrence rate of 5.6% after irradiation for Stage IB lesions and a rate of 7.7% for Stage IIA lesions. Johnsson,[4] using the Stockholm technique, had a local recurrence rate of 6% in Stage IB and 14% in Stage IIA at 2 years after treatment. Durrance and associates[5] reported a central recurrence rate of 1.9% at M. D. Anderson Hospital in 471 patients who had Stage I disease treated by radiotherapy and a rate of 5.6% in patients with Stage II disease. The authors noted an improvement with the advent of megavoltage equipment, a fact also reported by VilaSanta[6] and Halpin and associates.[7]

Table VI. Comparison of factors and sites of recurrence in patients with cervical cancer who had undergone radical hysterectomy

	Site of recurrence			
	Central	*Regional*	*Distant*	*Total*
Lesion size (cm):				
≤4	6	14	15	35
>4	3	13	17	33
Total	9	27	32	68
Lesion Stage:				
I	3	14	22	39
II	2	18	24	44
III	1	1	2	4
IV	0	1	0	1
Total	6	34	48	88
Previous treatment:				
Radiation	5	13	18	36
No radiation	4	27	37	68
Total	9	40	55	104
Cell type:				
Squamous	6	35	45	86
Other	3	5	10	18
Total	9	40	55	104
Lesion grade:				
1 and 2	2	6	8	16
3 and 4	7	34	47	88
Total	9	40	55	104
Positive lymph nodes:				
None	8	26	31	65
1	1	7	15	23
≥2	0	7	9	16
Total	9	40	55	104
Portion of vagina excised:				
None to 25%	3	3	8	14
≥26%	5	37	45	87
Total	8	40	53	101
Patient status:				
Alive	0	2	4	6
Dead	9	38	51	98
Total	9	40	55	104

In our series, lesion size was relatively unimportant with regard to site of recurrence. Graham and Graham,[8] Piver and Chung,[9] and Van Nagell and associates[10] have shown a relationship between lesion size and the incidence of lymph node metastasis and recurrence. The M. D. Anderson group[5] also reported problems with central recurrences in patients with large, "barrel-shaped" lesions who were treated radio-therapeutically, and they found that the addition of conservative hysterectomy significantly reduced these recurrences. However, in our series, twice as many central recurrences were noted in patients who had lesions of 4 cm or less in diameter compared with patients who had lesions greater than 4 cm in diameter. This finding is not unreasonable, because although larger lesions can make the operation more difficult technically, if the primary tumor mass is resected adequately and the extent of dissection is not compromised, central recurrences should not be more frequent when the lesions are larger.

The nine patients with "central recurrence alone" are the ones who are of great concern because they are most likely to signify technical failure of the treatment method. Five of these patients had previous irradiation as their primary treatment, and because of failure of this modality, they underwent operation. Of the four nonirradiated patients, none underwent postoperative pelvic irradiation. The initial stage of disease in two patients was unknown, three had Stage IB disease, two had Stage IIA disease, one had Stage IIB disease, and one had Stage IIIA disease. The average time until recurrence for these nine patients was 14 months, with a range of 4 to 30 months.

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Scanning electron microscopy of attachment of *Neisseria gonorrhoeae* colony phenotypes to surfaces of human genital epithelia

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Attachment of different gonococcal colony phenotypes to explants of human genital tract epithelium was studied by means of scanning electron microscopy and radioisotope-labeled gonococci. Heavily piliated organisms attached in greater numbers than nonpiliated organisms. Gonococci from transparent colony phenotypes attached in higher numbers than gonococci from opaque phenotypes to all tissues studied. Transitional cells from cervical tissues showing a gradual squamocolumnar transition demonstrated more gonococci attached per surface area than either endocervical or fallopian tube epithelium. Squamous epithelium showed the fewest number of attached gonococci. In all tissues, the attachment of the gonococcus was to the tips and surfaces of the microvilli. Gonococcal colony phenotypes as well as the length and location of the cervical transition zone may influence the progression of cervical gonococcal infection to invasive disease. (AM. J. OBSTET. GYNECOL. 138:818, 1980.)

THE IMPORTANT FIRST STEP in genital infections caused by *N. gonorrhoeae* is attachment of the gonococcus to the mucosal epithelium. Recent investigations[1-4] indicated that *N. gonorrhoeae* attach or adhere to a variety of human tissues and epithelial cells in vitro. It appears that several factors associated with colony phenotype may influence attachment including degree of piliation[5-7] and opacity or transparency of the colony. Cervical *N. gonorrhoeae* isolates vary in colony phenotype with menstrual cycle; opaque phenotypes are found at midcycle and transparent phenotypes occur at menstruation.[8, 9] Only transparent colony phenotypes are cultured from fallopian tube and cul-de-sac specimens obtained at laparoscopy.[10] These observations indicate that colony phenotype variation in attachment may be important in the initial *N. gonorrhoeae* attachment process and in the pathogenesis of *N. gonorrhoeae* salpingitis.

The purpose of this paper is to report on variations in attachment of gonococcal colony phenotypes to human female genital tract epithelia and to show visual documentation of the attachment phenomenon. Studies with radioisotope-labeled *N. gonorrhoeae* were done to analyze the cervical squamocolumnar junction attachment phenomenon.

Material and methods

Organisms. The laboratory strain of *Neisseria gonorrhoeae* F62 was used in the attachment studies. The colony phenotypes were passaged daily and maintained on modified GC agar base[8] supplemented with IsoVitaleX (Baltimore Biological Laboratory, Cockeysville, Maryland). Inocula were prepared from 16- to 20-hour-old cultures grown in 5% carbon dioxide at 37° C.

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Supported by Research Grants AI 15642 and HD 13462 from the National Institutes of Health and by United States Army Medical Research and Development Command DAMD17-79-C-9021

Received for publication April 9, 1980.

Revised July 25, 1980.

Accepted August 8, 1980.

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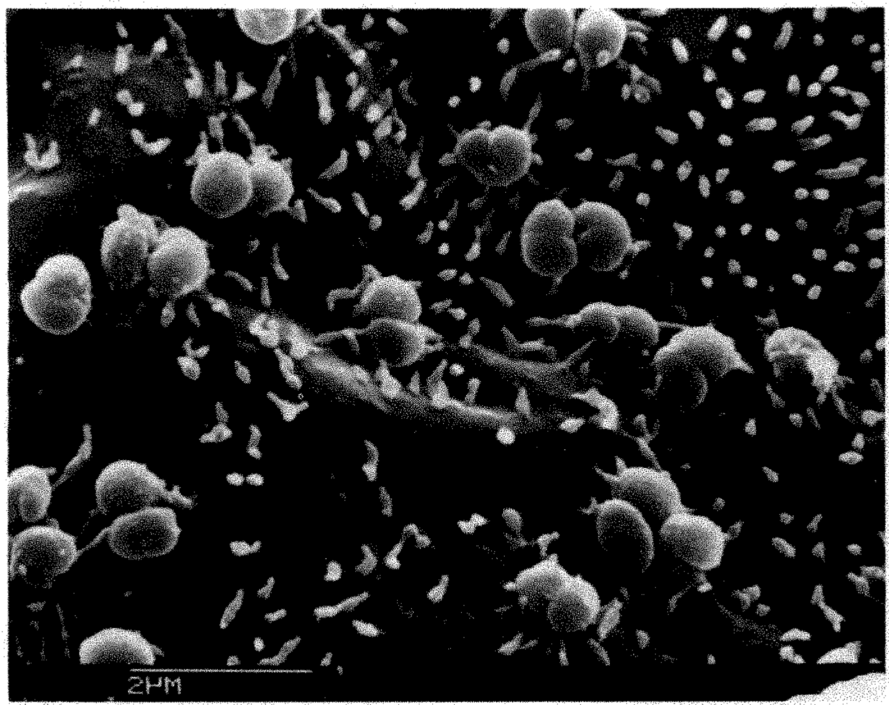

Fig. 1. Scanning electron micrograph of *N. gonorrhoeae* colony phenotype P++Tr attached to surface microvilli of an epithelial cell located in the transition zone of the squamocolumnar junction of the human cervix. The *N. gonorrhoeae* attachment appeared specific for the microvilli. (×12,000. Bar equals 2 μm.)

Radiolabeled gonococci were prepared as follows: Prior to inoculation, the surface of each 25 ml modified GC agar plate was spread with 0.075 ml of ^{3}H-adenine (0.5 mCi/ml) and allowed to dry; after inoculation the culture was incubated 16 to 20 hours in 5% carbon dioxide at 37° C.

The following nomenclature, modified from the classification of Kellogg and associates,[11] was used to describe the colony phenotypes: Kellogg colony type 2 has been found to be heavily piliated (P++) and to manifest opaque (Op) and transparent (Tr) variants. Kellogg colony type 3 has been found to be nonpiliated (P−) and of the opaque colony type. Kellogg colony type 4 has been found to be nonpiliated and transparent. Thus, four phenotypes were studied: P++Tr, P++Op, P−Tr, and P−Op.

Tissue explants. Fallopian tubes and cervical tissues were obtained from surgical specimens of women who underwent elective tubal ligation or complete hysterectomy performed at San Francisco General Hospital or at the University of California Medical Center. The tissues were obtained under the auspices of the University of California, San Francisco Committee on Human Research. The tissue pieces were placed into minimal essential medium (MEM) with VCN (Baltimore Biological Laboratory, Cockeysville, Maryland) and 10% fetal calf serum for transport to the laboratory. The tissues were gently processed to remove mucus and excess connective tissue and placed into Medium 199 (Microbiological Associates, Bethesda, Maryland) with antibiotics, glutamine, and bicarbonate buffer, pH 7.4. The tissues were maintained as explant cultures and incubated in 5% carbon dioxide, at 37° C. In general, the tissues were used in experiments within 24 hours of the time they were removed from the patient.

Attachment assays. Before an assay, each tissue was rinsed twice in Medium 199 without antibiotics. Accumulated mucus was removed by gentle washing with Medium 199 and suction with a Pasteur pipette. The tissues were carefully embedded in 1% Noble agar, made with Medium 199, so that only the epithelial surface was exposed.

The inoculum for each phenotype was prepared by suspending the *N. gonorrhoeae* growth in Medium 199 to a density of 50 Klett units (2 × 10^8 colony-forming units (CFU)/per milliliter). A further dilution of 1:5 was made and this inoculum (4 × 10^7 CFU/ml) was used immediately. A 7 ml volume was poured over the tissue and the assay dish was incubated on a shaker at 37° C, with 5% carbon dioxide for 30 minutes. After incubation, the inoculum was removed by aspiration

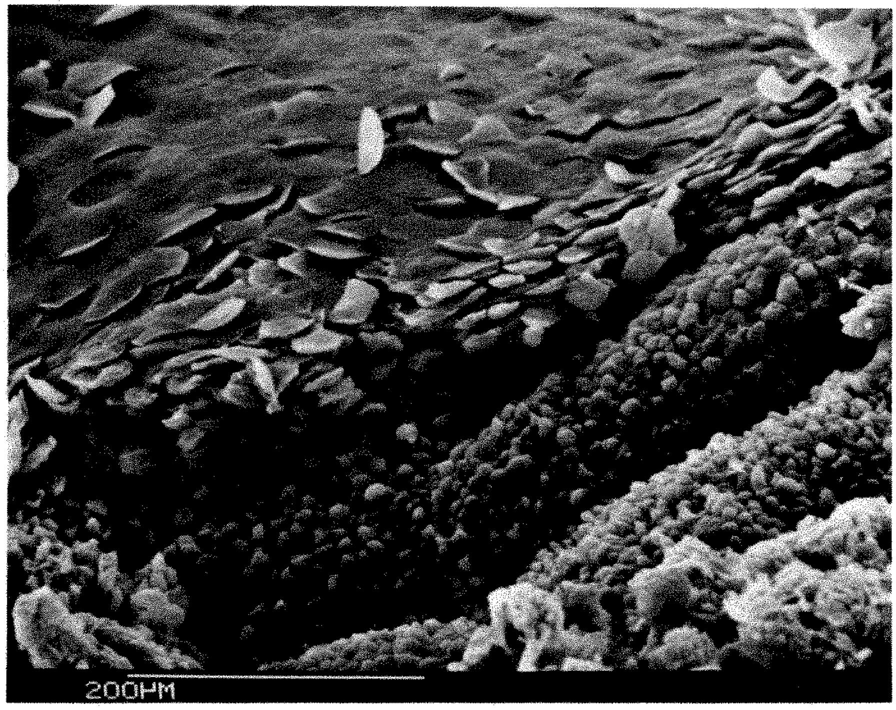

Fig. 2. Low-magnification scanning electron micrograph of human uterine cervical tissue at the squamocolumnar junction. The transition between squamous and columnar cells was abrupt. (× 190. Bar equals 200 μm.)

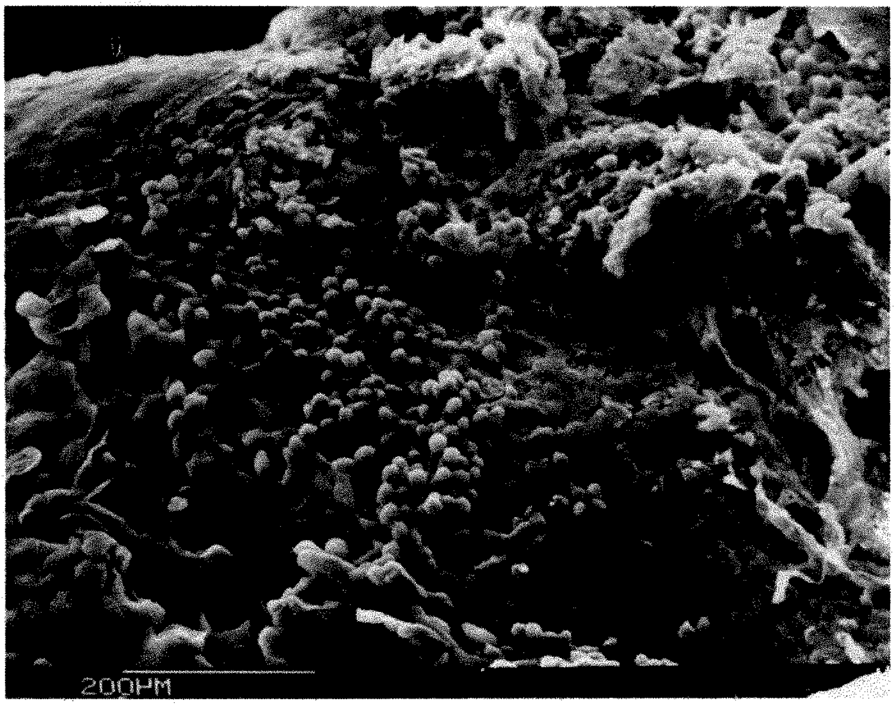

Fig. 3. Low-magnification scanning electron micrograph of human cervical tissue with wide squamocolumnar junction. The ectocervix is to the left and the endocervix is to the right (hidden by mucus and debris). Note transitional cells at center. (× 125. Bar equals 200 μm.)

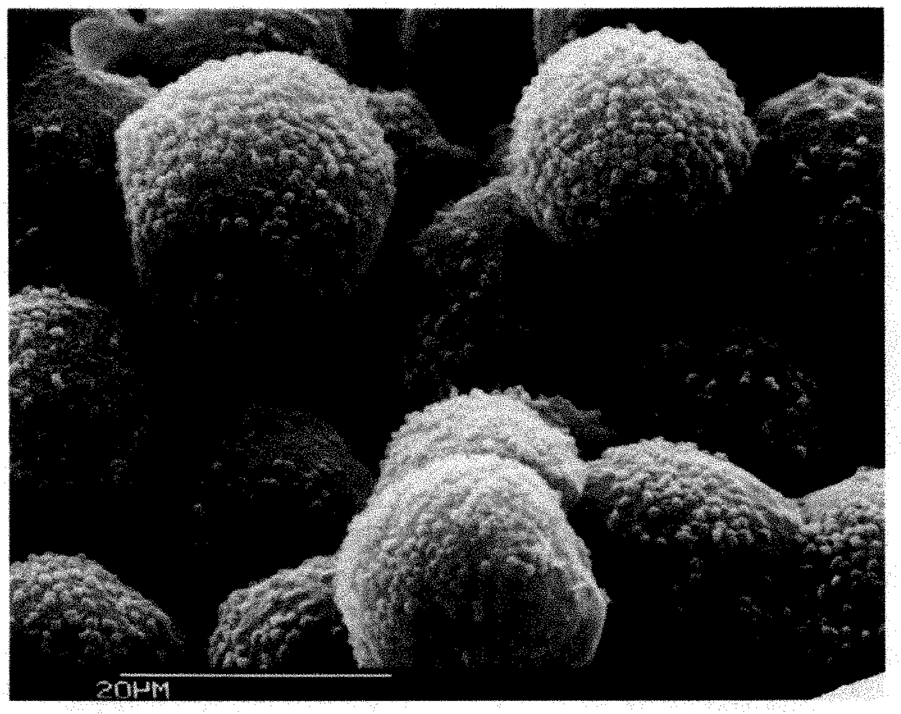

Fig. 4. Scanning electron micrograph of *N. gonorrhoeae* colony phenotype P++Tr attached in large numbers to transitional cells located in transition zone of human uterine squamocolumnar junction. (×1,750. Bar equals 20 μm.)

and the tissues were rinsed twice with Medium 199 and immediately fixed for scanning electron microscopy. Seven cervical and seven fallopian tube tissues, each from a different patient, were studied. Each *N. gonorrhoeae* colony phenotype was tested at least three times.

Scanning electron microscopy (SEM). The tissues were fixed in 3% glutaraldehyde (Sigma Chemical Co., St. Louis, Missouri) buffered with 0.1M sodium cacodylate (Sigma Chemical Co.), pH 7.0, for 10 hours at room temperature. The specimens were dehydrated in increasing concentrations of ethanol with 10-minute exchanges, dried in a Bomar critical point dryer, sputter coated with gold (Hummer sputter coater), and viewed with a Cambridge S150 scanning electron microscope at an accelerating voltage of 20 kV.

Radiolabeled attachment assays. Inocula of gonococci from the ^{3}H-labeled colony phenotypes were prepared and used as described above. At the conclusion of the assay, the tissues were rinsed with Medium 199 and serial cross sections of pieces of equal epithelial surface areas were obtained. These pieces were placed into Aquasol scintillation fluid (New England Nuclear, Boston, Massachusetts) for ^{3}H count determination.

Results

Explant tissues. Grossly, the cervical explant tissues remained intact and appeared viable after 6 to 8 days' incubation. Sections examined by light microscopy showed layered squamous cells (ectocervix) and intact mucus-secreting cells interspersed among ciliated cells (endocervix). After incubation for 3 to 8 days the cervical explants produced a grossly visible amount of mucus; some explants were nearly enveloped in mucus which, at times, was difficult to rinse off. Because of the large amounts of mucus seen with prolonged incubation, the attachment and SEM studies were done within 18 hours of the time the specimens were taken from the patients and mucus was rinsed from the specimens prior to use.

The fallopian tube explant tissues remained viable with vigorously beating cilia for more than 14 days. The gross and light microscopy appearances were similar to those previously reported by Magee and coworkers.[12]

SEM of cervical explants. The squamous cells of the ectocervix distant from the squamocolumnar junction had prominent surface microridges (microplicae). However, at or near the squamocolumnar junction, the cells appeared to have fewer microplicae and more prominent microvilli. This change in surface characteristics appeared to be associated with enhanced *N. gonorrhoeae* attachment. The *N. gonorrhoeae* appeared to attach much more frequently to squamous cells near the squamocolumnar junction and the attachment ap-

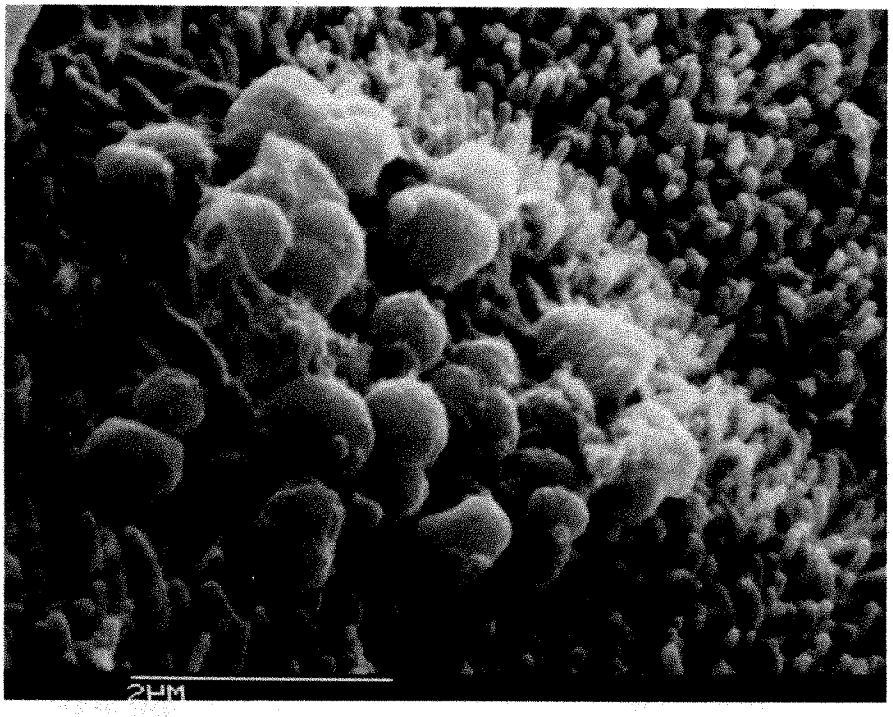

Fig. 5. Scanning electron micrograph of *N. gonorrhoeae* colony phenotype P++Tr attached to the surface of human endocervical cells. Much of the attachment appears to be associated with microvilli. (×16,000. Bar equals 2 µm.)

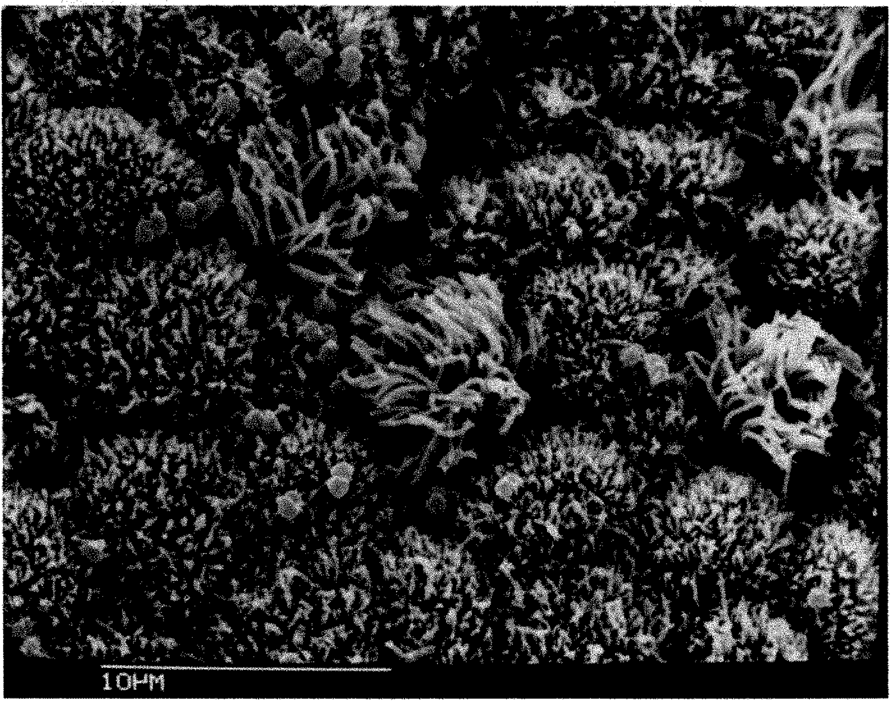

Fig. 6. Scanning electron micrograph of *N. gonorrhoeae* colony phenotype P++Tr attached to surface of several nonciliated columnar epithelial cells of human fallopian tube. After 30 minutes' incubation, the *N. gonorrhoeae* were found attached singly and in pairs. (×3,700. Bar equals 10 µm.)

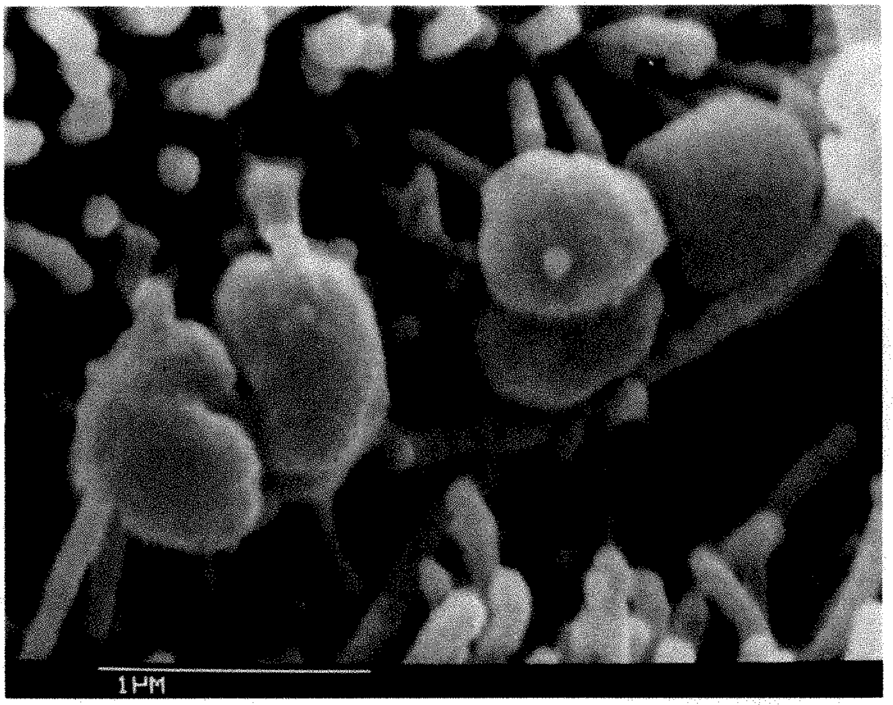

Fig. 7. Scanning electron micrograph of *N. gonorrhoeae* attached to microvilli of columnar cells of fallopian tube epithelium. The microvilli appear firmly attached to the organism. (×34,000. Bar equals 1 μm.)

peared to be directly associated with microvilli (Fig. 1).

A cervical explant tissue squamocolumnar junction is depicted in Fig. 2. The breadth of the transition zone between squamous and columnar cells was variable from specimen to specimen. In some the transition was abrupt (about three cells wide), while in others it was more gradual (about 20 cells wide). Rounded, transitional cells were noted at the squamocolumnar junction (Fig. 3). Very large numbers of gonococci were found attached to the surfaces of the transitional cells (Fig. 4). When high magnification was used, the attachment of *N. gonorrhoeae* to the transitional cells was associated with the numerous microvilli and surface ridges.

In the endocervical area, the columnar epithelial cell microvilli were longer and more numerous than those on the squamous cells or the cells of the squamocolumnar junction. The *N. gonorrhoeae* were found attached singly and in groups on the surface microvilli of the columnar cells (Fig. 5).

SEM of fallopian tube explants. The surface of the normal fallopian tube columnar epithelium was covered by a layer of nonciliated cells interspersed with ciliated cells. The nonciliated cells were thickly covered by microvilli. When gonococci were added to the system, the organisms were found attached only to the nonciliated cells and the attachment was to the microvilli (Fig. 6). At high magnification, the association of the *N. gonorrhoeae* and the microvilli was visualized (Fig. 7). Several microvilli were seen to be attached to each gonococcus and occasionally the microvilli appeared to wrap across the surface of the bacteria.

Attachment of *N. gonorrhoeae* colony phenotypes. In the cervical explant systems, isogenic piliated and nonpiliated, opaque and transparent variants of F62 were tested for their ability to attach. Each of the four colony phenotypes tested (P++Tr, P++Op, P−Tr, and P−Op) attached to endocervical tissue; however, by SEM, the piliated organisms attached in fourfold to tenfold higher numbers than nonpiliated variants. Relatively few of the *N. gonorrhoeae* were seen attached to squamous cells of the ectocervix. Organisms were predominantly seen on squamous cells very near the squamocolumnar junction.

The results of four attachment assays of ^{3}H-labeled piliated, isogenic transparent and opaque colony variants to cervical tissue are presented in Fig. 8. Organisms from both P++Op and P++Tr colonies of F62 attached in relatively low numbers (1.0% inoculum bound) to the squamous epithelial cells of the ectocervix (0.5 cm from the squamocolumnar junction). The numbers of attached gonococci from P++Op colonies did not increase at or near the squamocolumnar junc-

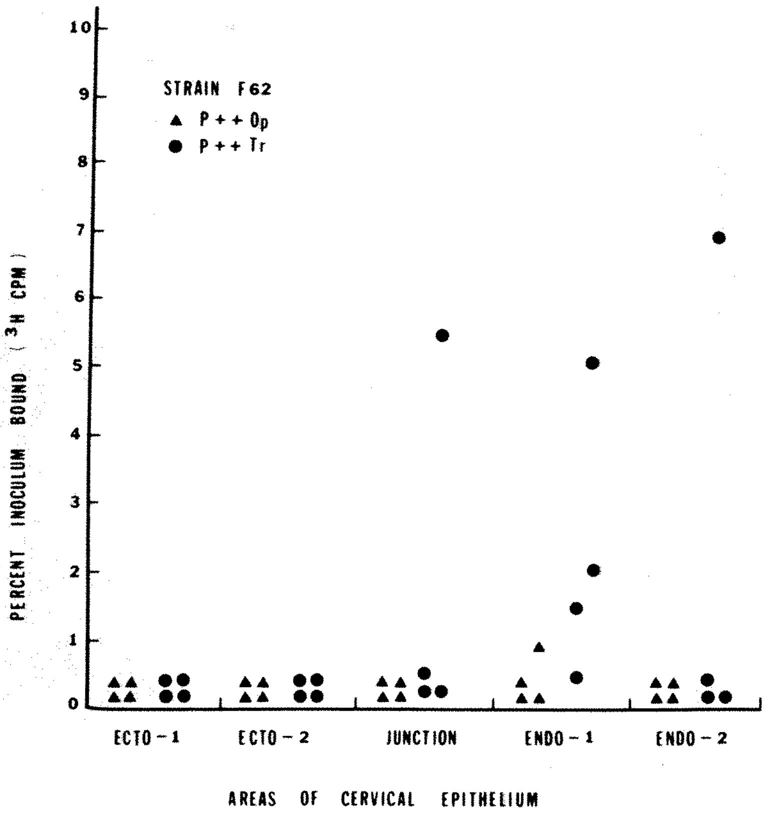

Fig. 8. The attachment of intrinsically radiolabeled *N. gonorrhoeae* from piliated transparent (P++Tr) and piliated opaque (P++Op) colony phenotypes to human cervical tissue. The level of attachment of both phenotypes to ectocervical epithelium was less than 1%. Piliated transparent phenotypes attached to junctional and endocervical epithelium in substantially higher numbers than piliated opaque phenotypes.

tion. However, a marked increase in the attachment of P++Tr variants was noted at the squamocolumnar junction and in the endocervical areas. Confirmatory, but less quantitative, results were noted with SEM visualization.

In the fallopian tube explant system, SEM analysis indicated that the *N. gonorrhoeae* from P++Tr colonies were attached to the epithelium tenfold to 100-fold more frequently than the P++Op phenotypes.

In the experiments with cervical explants, the presence of mucus sometimes obscured the epithelial surfaces and made scanning visualization difficult. However, *N. gonorrhoeae* from each of the four colony phenotypes were frequently found adherent to or entangled in mucus strands (Fig. 9). The nonpiliated (P−Op and P−Tr) colony phenotypes were most frequently associated with the cervical mucus.

Comment

In women, the cervix is the primary site of most *N. gonorrhoeae* mucosal infections, and approximately 15% of untreated cervical infections progress to involvement of the fallopian tubes. The first event in any mucosal infection is the attachment of the pathogen to mucosa. The pathogenesis of a mucosal infection is dependent upon both host and organism factors which promote this attachment. In this study, we examined the attachment of gonococcal colony phenotypes to human uterine cervix and fallopian tube mucosa in order to better understand the pathogenesis of this human infection.

By both SEM and radiolabeled *N. gonorrhoeae* attachment assay, very few gonococci from any of the four colony phenotypes tested were found to attach to squamous cells of the ectocervix. These data are in contrast to the observations of Evans.[13] In a transmission electron microscopy study done of cervical biopsy tissues of patients with gonorrhea, he found that the predominant attachment of the organism was to the squamous epithelial cells of the ectocervix, rather than to cells of the transition zone or to columnar cells of the endocervix. Our data indicate that *N. gonorrhoeae* do not attach efficiently to squamous epithelial cells but do attach in large numbers to cells of the transition zone

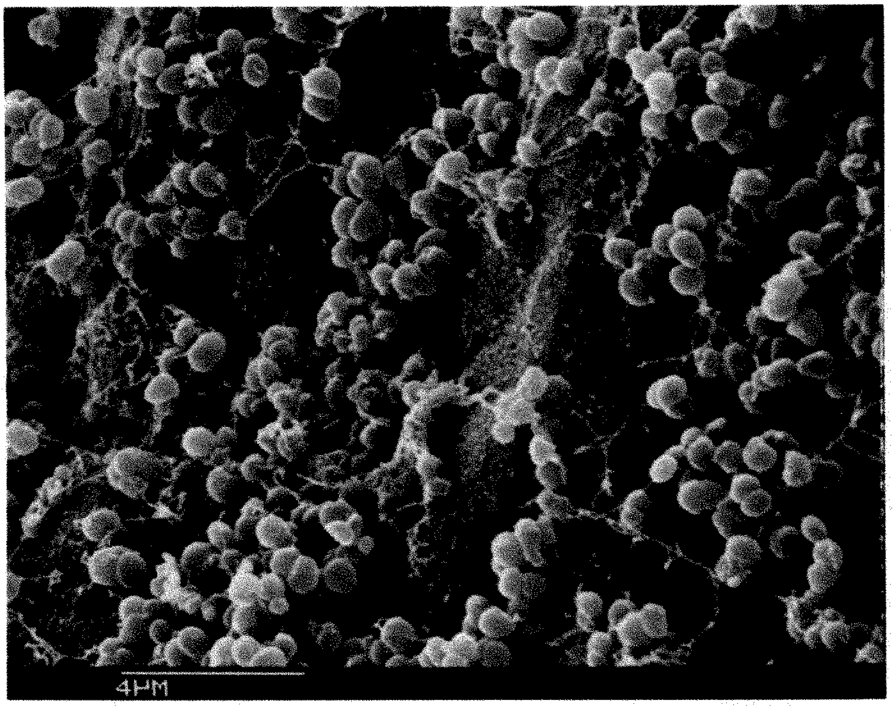

Fig. 9. Scanning electron micrograph of *N. gonorrhoeae* colony phenotype P−Tr entangled in cervical mucus strands. Clumps of *N. gonorrhoeae* appeared to be resting on or possibly adherent to the cervical basement membrane. (×5,750. Bar equals 4 μm.)

and the columnar epithelial cells. Our conclusion is consistent with light microscopy observations published in the 1890s[14, 15] and reviewed by Harkness in 1948.[16]

Piliated gonococci, by SEM, were more frequently attached to ectocervical cells than nonpiliated gonococci. The ectocervical cells showed numerous microplicae which occasionally contained short, stubby microvillus-like projections. The few gonococci which attached to squamous cells were attached at microplical ridges frequently in association with villus-like projections.

Microplicae have not been previously known to play a role in cellular attachment of gonococci. The role of microplicae in surface function of squamous epithelial cells is not clear. It has been suggested that microplicae differ from microvilli and are the remnants of interdigitating and adhesive cell processes of the epithelial cells formed at deeper layers.[17] Microplicae are covered by a polyamine glycocalyx and have, subjacent to the plasmalemma, a disorderly array of fine electron-dense fibers. Microplicae thus differ from microvilli in function and architecture. Variation in attachment of gonococci to squamous cells may be partially a function of these differences.

Variation in the width of the squamocolumnar junction had a striking influence on attachment of *N. gonorrhoeae*. When the transition zone was gradual, there was a larger number of organisms attached in this area as compared to abrupt transition zones. By SEM, the transitional cells showed rudimentary microplicae and scattered microvilli. The number of attached gonococci to transitional cells was much higher than the number seen on the surfaces of both endocervical and ectocervical cells where the microvilli and microplicae, respectively, are more fully developed. Membrane properties of transitional cells may be particularly important for attachment of gonococci to this area.

In the uterine cervix, there is a normal anatomic variation in the length of the transitional zone.[18] Similarly, the presence and amount of columnar epithelium extending onto the ectocervical surface show anatomic variation.[19] It may be that the more gradual transition zone and the presence and amount of ectocervical columnar epithelium influence host susceptibility to gonorrhea by providing increased sites for bacterial attachment.

N. gonorrhoeae appeared to attach very effectively to endocervical epithelium and to fallopian tube epithelium. The *N. gonorrhoeae* attached to tips and surfaces of microvilli of the columnar cells of the endocervical and fallopian tube mucosa. The organism was often seen wrapped in projections of microvilli. Previous transmission electron microscopy studies of fallopian tube explants have demonstrated gonococci held in cuplike projections of microvilli.[12]

Although organisms from both opaque and transparent colony phenotypes were attached, piliated *N. gonorrhoeae* attached in higher numbers than gonococci from isogenic nonpiliated colony variants. The role of pili in attachment and virulence of *N. gonorrhoeae* has been extensively studied and pili are considered to be one of the prime factors in attachment.[5–7]

Gonococci from transparent colonies attached in higher numbers than gonococci from isogenic opaque colonies of comparable piliation. The greater attachment of organisms of transparent colony phenotypes to the cervical and follopian tube tissues is a potentially important observation in understanding the pathogenesis of *N. gonorrhoeae* infections. It appears that the gonococci from transparent colonies may have a factor(s) other than pili which are important in attachment and tissue tropism. Only the transparent colony phenotypes have been found in fallopian tube and cul-de-sac specimens obtained at laparoscopy from women with salpingitis. This suggests that the transparent phenotype may be more important in the invasive process of salpingitis.[10] Although transparent phenotypes are associated with invasive disease, opaque colony phenotypes are the predominant variants found in urethral exudates of infected men and are presumed to be the phenotypes routinely transmitted to women.[8, 9] *N. gonorrhoeae* shift colony phenotype in vitro and presumably can also express different phenotype characteristics in vivo.[9] Thus, a woman inoculated with one predominant phenotype may yield a different phenotype on subsequent culture. Additionally, it is possible that there is cyclic variation in membrane properties of cervical epithelium which determine attachment affinities for different gonococcal colony phenotypes.

In summary, attachment of gonococci to the mucosa of human cervical explant tissue varies with the epithelial cell type examined. The gonococci attached in scant numbers to the microplicae of squamous epithelium but attached in higher numbers to transitional cells of the cervix, where they are associated with both microplicae and microvilli. High numbers of gonococci attached to the microvilli of both columnar endocervical epithelial cells and fallopian tube epithelial cells. *N. gonorrhoeae* from transparent colonies attached in greater numbers than isogenic opaque variants to both cervical and fallopian tube epithelium.

We would like to acknowledge the excellent technical advice and consultations of Dr. John Long and Mr. Jerry Morgan of the Scanning Electron Microscopy Laboratory at the University of California, San Francisco.

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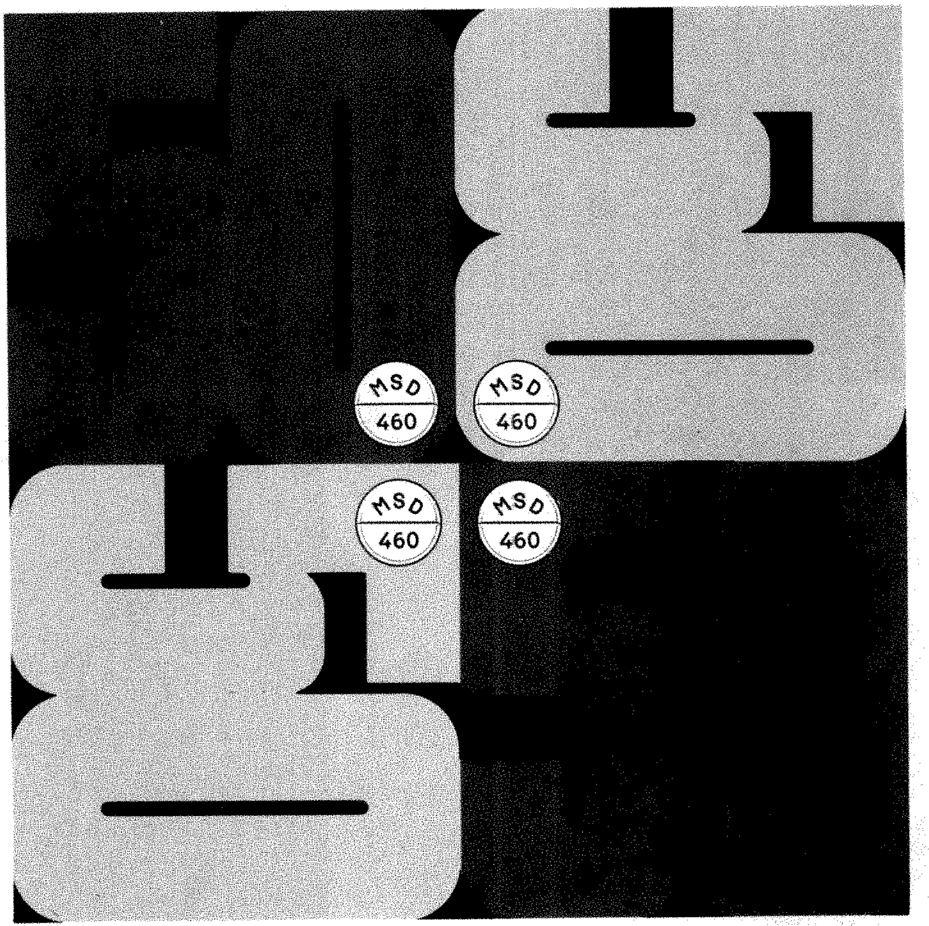
for neurogenic atony of the urinary bladder with retention, higher titrated dosages may be needed (up to 200 mg/day)



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when higher titrated dosages are indicated

After titration, dosages as high as 50 mg t.i.d. or q.i.d. have been effectively employed in neurogenic atony of the urinary bladder as well as for the treatment of postoperative and postpartum nonobstructive (functional) urinary retention.

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URECHOLINE should not be used when the strength or integrity of the gastrointestinal or bladder wall is in question or in the presence of mechanical obstruction. If necessary, the effects of the drug can be abolished promptly by atropine.

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for neurogenic atony of the urinary bladder with retention, higher titrated dosages may be needed (up to 200 mg/day)

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Warnings: *The sterile solution is for subcutaneous use only. It should never be given intramuscularly or intravenously.* Violent symptoms of cholinergic overstimulation, such as circulatory collapse, fall in blood pressure, abdominal cramps, bloody diarrhea, shock, or sudden cardiac arrest are likely to occur if the drug is given by either of these routes. Although rare, these same symptoms have occurred after subcutaneous injection, and may occur in cases of hypersensitivity or overdosage.

Precautions: Special care is required in patients receiving ganglion blocking compounds because a critical fall in blood pressure may occur; usually, severe abdominal symptoms appear before there is such a fall in blood pressure. In urinary retention, if the sphincter fails to relax as the drug contracts the bladder, urine may be forced up the ureter into the kidney pelvis; if there is bacteriuria, this may cause reflux infection.

Adverse Reactions: Abdominal discomfort, salivation, flushing of the skin ("hot feeling"), sweating. Large doses more commonly result in effects of parasympathetic stimulation, such as malaise, headache, sensation of heat about the face, flushing, colicky pain, diarrhea, nausea and belching, abdominal cramps, borborygmi, asthmatic attacks, and fall in blood pressure.

Atropine is a specific antidote. The recommended dose for adults is 0.6 mg (1/100 grain). The recommended dosage in infants and children up to 12 years of age is 0.01 mg/kg repeated every two hours as needed until the desired effect is obtained, or adverse effects of atropine preclude further usage. The maximum single dose should not exceed 0.4 mg. Subcutaneous injection of atropine is preferred except in emergencies when the intravenous route may be employed. When Injection URECHOLINE is used, a syringe of atropine sulfate should always be available.

How Supplied: Tablets, containing 5 mg, 10 mg, 25 mg, or 50 mg bethanechol chloride each, in bottles of 100 and single-unit packages of 100; Injection, 5 mg per ml, is a clear, colorless solution, and is supplied in boxes of 6 × 1-ml vials.

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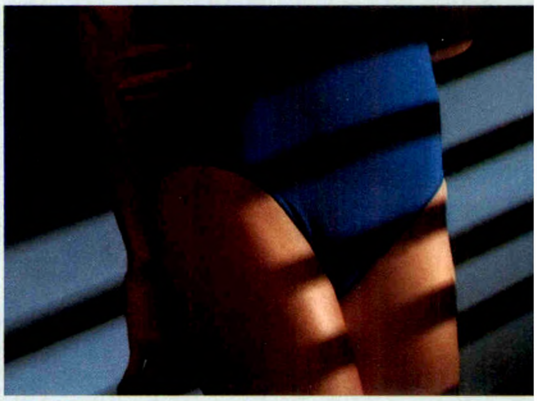

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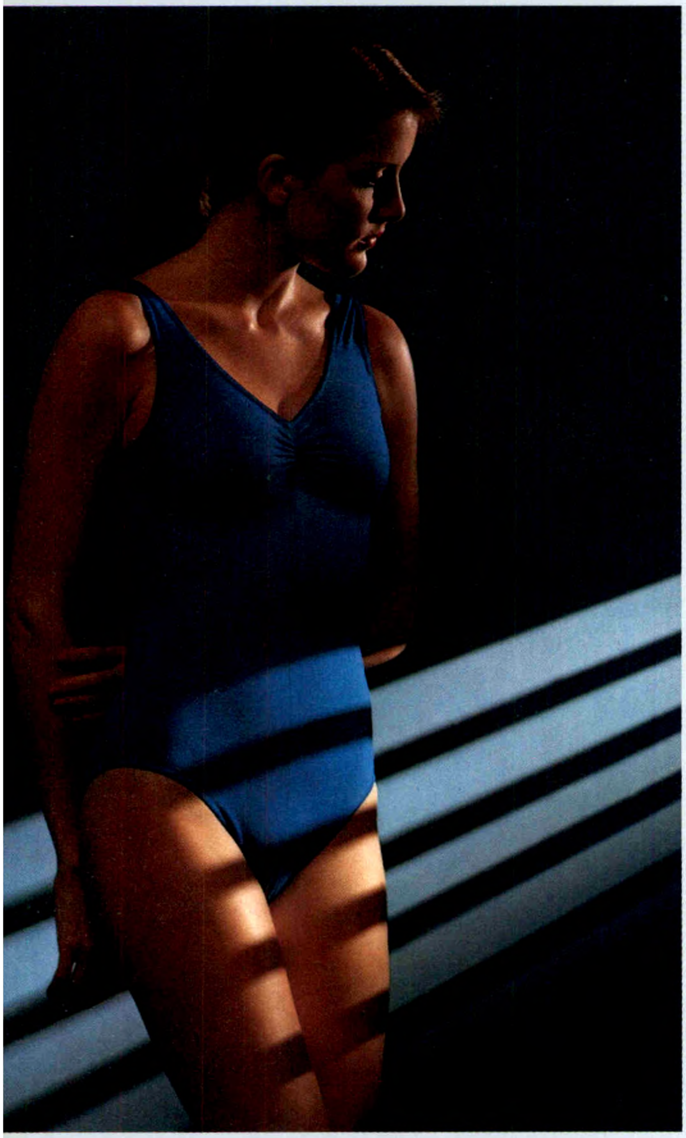


NEW

275mg TABLETS

ANAPROX®

(NAPROXEN SODIUM)

A new th[illegible]rapeutic alt[illegible]rnative

ORRHEA

A NON-NARCOTIC, SINGLE-ENTITY ORAL ANALGESIC*

New Anaprox® (naproxen sodium) is an effective, mid-range analgesic that has been proven to be useful in the treatment of primary dysmenorrhea. Anaprox represents a new therapeutic alternative for obstetricians/gynecologists who frequently encounter primary dysmenorrhea in practice.

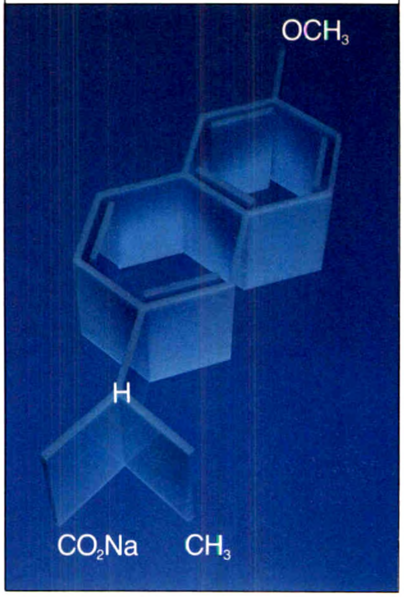
FULL THERAPEUTIC EFFECT IN ONE HOUR

With Anaprox, full therapeutic effect is reached within one hour. Peak plasma levels are attained in 1-2 hours. Anaprox also offers a longer half-life than other non-hormonal agents indicated for dysmenorrhea. This helps sustain relief of painful dysmenorrhea from dose to dose, whether administered t.i.d. or q.i.d.

PROVIDES FAST, LONG-LASTING RELIEF OF PAIN AND UTERINE CRAMPING

Rapid absorption and fast onset of action help assure rapid relief of the distressing symptoms of dysmenorrhea —pain and uterine cramping.

Anaprox
Chemical Structure



AN EFFECTIVE INHIBITOR OF PROSTAGLANDIN SYNTHETASE AND UTERINE CONTRACTILITY

Anaprox, a member of the arylacetic acid class of drugs, is an effective inhibitor of prostaglandin synthetase. In patients studied, it has been shown that Anaprox significantly reduces the level of prostaglandins in the uterus, which is accompanied by significant reduction in uterine contractions. Reduced contractility results in decreased painful cramping of dysmenorrhea.

WELL TOLERATED BY MOST PATIENTS

Anaprox was well tolerated in most patients studied. In fact, studies showed a lower incidence of GI and CNS side effects when it was compared with aspirin. With Anaprox, the most common adverse reactions were mild GI and CNS side effects, which occurred in approximately 10% and 11%, respectively. Use with caution in patients with impaired renal function. Because of adverse eye changes with NSAIs, periodic ophthalmic studies should be carried out. Peripheral edema has been observed in some patients. Because of its antipyretic properties, Anaprox may mask the signs and symptoms of infection. Before prescribing, please see warnings, precautions, and adverse reactions sections of full Prescribing Information.

Before initiating Anaprox therapy, it should be deter-

***Anaprox is a nonsteroidal anti-inflammatory agent, which has been developed as an analgesic and possesses antipyretic properties.**

Please see full Prescribing Information on last pag

DYSMEN

mined that the patient is not pregnant.

Anaprox should not be used concomitantly with the related drug Naprosyn® (naproxen), since both circulate in plasma as the naproxen anion and have the same side effects.

EXTENSIVE CLINICAL DOCUMENTATION

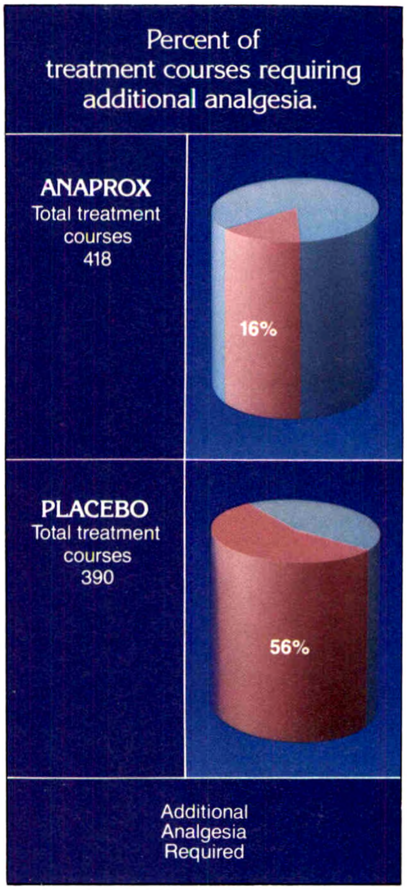
Anaprox has been the subject of extensive clinical investigation in dysmenorrhea, having been studied in 212 patients over 496 menstrual cycles.

EFFICACY DEMONSTRATED BY RELIEF OF MENSTRUAL PAIN

In eleven double-blind parallel or cross-over studies, and one single-blind study, involving 212 patients over 496 treatment courses, patients in 63% of the treatment courses derived "substantial to complete relief" and 11% experienced "mild to substantial relief" with Anaprox. Researchers concluded that "... a significant and beneficial effect of Anaprox on menstrual pain can be expected in about 75% of the treatment courses."

EFFICACY DEMONSTRATED BY REDUCED NEED FOR ADDITIONAL ANALGESIA

The eleven double-blind comparison studies with placebo were conducted over 808 treatment courses. In 418 Anaprox treatment courses, only 16% required additional pain medication. In 390 placebo treatment courses, however, additional analgesia was required in 56% of the treatment courses.



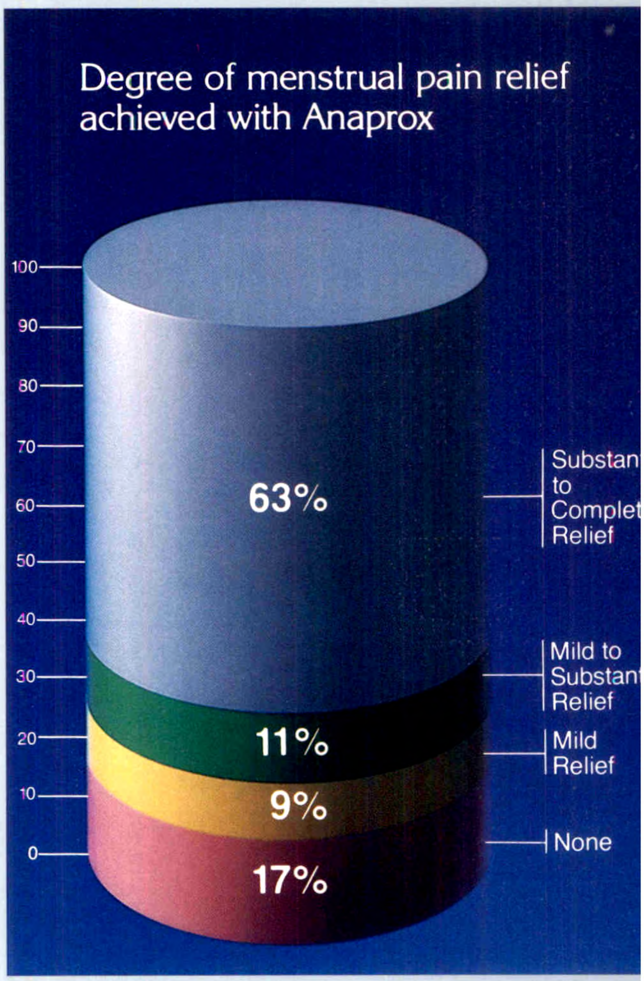
EFFICACY DEMONSTRATED BY LESS DAILY ACTIVITY INTERFERENCE

Continuation of patients' usual daily activities during painful menstruation was evaluated in a double-blind study involving 64 patients given Anaprox or placebo. Scores were totaled over three treatment courses. The study showed conclusively that those patients treated with Anaprox experienced substantially less interference with usual daily activities than those treated with placebo.

EFFICACY DEMONSTRATED BY DECREASED INTRAUTERINE PRESSURE

Anaprox efficacy in dysmenorrhea has been further documented by measured decrease in uterine pressure. Measurement was conducted using a microballoon-tipped catheter introduced transcervically into the uterine cavity and connected to a recording system. Patients treated with Anaprox showed a significant decrease in resting pressure, amplitude, and frequency of uterine contractions.

ORRHEA



Degree of menstrual pain relief achieved with Anaprox

NEW

275mg TABLET

ANAPROX®

(NAPROXEN SODIUM

A new therapeutic alternativ

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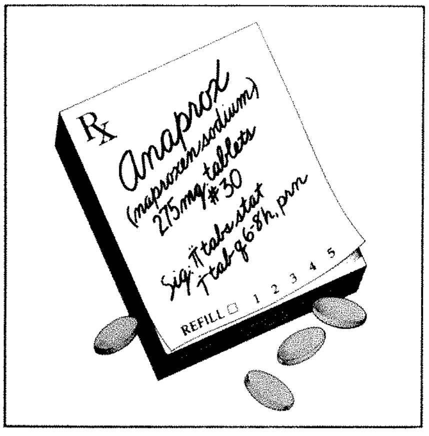
FOR DYSMENORRHEA

NEW ANAPROX®

275 mg tablets

(NAPROXEN SODIUM)



Description: ANAPROX filmcoated tablets for oral administration each contain 275 mg of naproxen sodium, which is equivalent to 250 mg naproxen with 25 mg (about 1 mEq) sodium. It is related to the arylacetic acid class of drugs. ANAPROX is a non-narcotic analgesic agent with anti-inflammatory and antipyretic actions. These properties have been demonstrated in classical animal test systems.

The chemical name of naproxen sodium is (−)-sodium 2-(6-methoxy-2-naphthyl) propionate.

Naproxen sodium is a white to creamy white, crystalline solid, freely soluble in water.

Clinical Pharmacology: Naproxen sodium is rapidly and completely absorbed from the gastrointestinal tract. After administration of naproxen sodium, peak plasma levels of naproxen anion are attained at 1-2 hours. The mean biological half-life of the anion in humans is approximately 13 hours, and at therapeutic levels it is greater than 99% albumin bound. Approximately 95% of the dose is excreted in the urine, primarily as naproxen, 6-0-desmethyl naproxen or their conjugates. The rate of excretion has been found to coincide closely with the rate of drug disappearance from the plasma. The drug does not induce metabolizing enzymes. Naproxen anion inhibits prostaglandin synthesis but beyond this its mode of action is unknown.

The drug was studied in patients with mild to moderate pain, and pain relief was obtained within 1 hour. It is not a narcotic and is not a CNS-acting drug. Controlled double-blind studies have demonstrated the analgesic properties of the drug in, for example, post-operative, post-partum, orthopedic and uterine contraction pain and dysmenorrhea. In dysmenorrheic patients, the drug reduces the level of prostaglandins in the uterus, leading to a reduction in the frequency and severity of uterine contractions. Analgesic action has been shown by such measures as reduction of pain intensity scores, increase in pain relief scores, decrease in numbers of patients requiring additional analgesic medication, and delay in time for required remedication. The analgesic effect has been found to last for up to 7 hours.

The drug was studied in patients with rheumatoid arthritis and osteoarthritis. It is not a corticosteroid. Improvement in patients treated for rheumatoid arthritis has been demonstrated by a reduction in joint swelling, a reduction in pain, a reduction in duration of morning stiffness, a reduction in disease activity as assessed by both the investigator and patient, and by increased mobility as demonstrated by a reduction in walking time.

In patients with osteoarthritis, the therapeutic action of the drug has been shown by a reduction in joint pain or tenderness, an increase in range of motion in knee joints, increased mobility as demonstrated by a reduction in walking time, and improvement in capacity to perform activities of daily living impaired by the disease.

In clinical studies in patients with rheumatoid arthritis and osteoarthritis, the drug has been shown to be comparable to aspirin in controlling the aforementioned measures of disease activity, but the frequency and severity of the milder gastrointestinal adverse effects (nausea, dyspepsia, heartburn) and nervous system adverse effects (tinnitus, dizziness, light-headedness) were less than in the aspirin-treated patients. It is not known whether the drug causes less peptic ulceration than aspirin.

The drug may be used safely in combination with gold salts and/or corticosteroids; however, in controlled clinical trials, when added to the regimen of patients receiving corticosteroids it did not appear to cause greater improvement over that seen with corticosteroids alone. Whether the drug could be used in conjunction with partially effective doses of corticosteroid for a "steroid-sparing" effect has not been adequately studied. When added to the regimen of patients receiving gold salts, the drug did result in greater improvement. Its use in combination with salicylates is not recommended because data are inadequate to demonstrate that the drug produces greater improvement over that achieved with aspirin alone. Further, there is some evidence that aspirin increases the rate of excretion of the drug.

Generally, improvement due to the drug has not been found to be dependent on age, sex, severity or duration of disease.

In ^{51}Cr blood loss and gastroscopy studies with normal volunteers, daily administration of 1100 mg of ANAPROX (naproxen sodium) has been demonstrated to cause statistically significantly less gastric bleeding and erosion than 3250 mg of aspirin.

Indications and Usage: ANAPROX® (naproxen sodium) is indicated in the relief of mild to moderate pain and for the treatment of primary dysmenorrhea.

It is also indicated for the treatment of the signs and symptoms of rheumatoid arthritis and osteoarthritis.

The safety and effectiveness of the drug in children have not been established.

Contraindications: Naproxen sodium is contraindicated in patients who have shown hypersensitivity to it. Because the potential exists for cross-sensitivity reactions, it should not be given to patients in whom aspirin or other non-steroidal, anti-inflammatory drugs induce the syndrome of asthma, rhinitis, or urticaria.

Warnings: Gastrointestinal bleeding, sometimes severe, and occasionally fatal, has been reported in patients receiving the drug. Among 960 patients treated for rheumatoid arthritis or osteoarthritis during the course of clinical trials in the United States (260 treated for more than two years), 16 cases of peptic ulceration were reported. More than half were on concomitant corticosteroid and/or salicylate therapy and about a third had a prior history of peptic ulcer. Gastrointestinal bleeding, including nine potentially serious cases, was also reported in this population. These were not always preceded by premonitory gastrointestinal symptoms. Although most of the patients with serious bleeding were receiving concomitant therapy and had a history of peptic ulcer disease, it should be kept in mind that the drug also has the potential for causing gastrointestinal bleeding on its own. Therefore, it should be administered to patients with active gastric and duodenal ulcers only under close supervision.

Precautions: General: *ANAPROX®* (NAPROXEN SODIUM) SHOULD NOT BE USED CONCOMITANTLY WITH THE RELATED DRUG *NAPROSYN®* (NAPROXEN) SINCE THEY BOTH CIRCULATE IN PLASMA AS THE NAPROXEN ANION.

Because of adverse eye findings in animal studies with drugs of this class, it is recommended that ophthalmic studies be carried out within a reasonable period of time after starting therapy and at periodic intervals thereafter if the drug is to be used for an extended period of time.

In chronic studies in laboratory animals, the drug has caused nephritis. Glomerular nephritis, interstitial nephritis and nephrotic syndrome have been reported. Since the drug is eliminated to a large extent from the body by urinary excretion via glomerular filtration, this drug should be used with great caution in patients with significantly impaired renal function and the monitoring of serum creatinine and/or creatinine clearance is advised in these patients. In clinical trials a few patients developed mild elevations in BUN accompanied by no other signs and symptoms.

If steroid dosage is reduced or eliminated during therapy, the steroid dosage should be reduced slowly and the patients must be observed closely for any evidence of adverse effects, including adrenal insufficiency and exacerbation of symptoms of arthritis.

Patients with initial hemoglobin values of 10 grams or less who are to receive long-term therapy should have hemoglobin values determined frequently.

Peripheral edema has been observed in some patients. Since each naproxen sodium tablet contains approximately 25 mg (about 1 mEq) of sodium, this should be considered in patients whose overall intake of sodium must be markedly restricted. It is possible that patients with questionable or compromised cardiac function may be at a greater risk when taking the drug.

Information for Patients: Caution should be exercised by patients whose activities require alertness if they experience drowsiness, dizziness, vertigo or depression during therapy with the drug.

Drug Interactions: *In vitro* studies have shown that naproxen anion, because of its affinity for protein, may displace from their binding sites other drugs which are also albumin-bound. Theoretically, the naproxen anion itself could likewise be displaced. Short-term controlled studies failed to show that taking the drug significantly affects prothrombin times when administered to individuals on coumarin-type anticoagulants. Caution is advised nonetheless, since interactions have been seen with other nonsteroidal agents of this class. Similarly, patients receiving the drug and a hydantoin, sulfonamide or sulfonylurea should be observed for signs of toxicity to these drugs.

Probenecid given concurrently increases naproxen anion plasma levels and extends its plasma half-life significantly.

Drug/Laboratory Test Interactions: The drug may decrease platelet aggregation and prolong bleeding time. This effect should be kept in mind when bleeding times are determined.

The administration of the drug may result in increased urinary values for 17-ketogenic steroids because of an interaction between the drug and/or its metabolites with m-dinitro-benzene used in this assay. Although 17-hydroxy-corticosteroid measurements (Porter-Silber test) do not appear to be artifactually altered, it is suggested that therapy with the drug be temporarily discontinued 72 hours before adrenal function tests are performed.

The drug may interfere with some urinary assays of 5-hydroxy indoleacetic acid (5HIAA).

Carcinogenesis: A two-year study was performed in rats to evaluate the carcinogenic potential of the drug. No evidence of carcinogenicity was found.

Pregnancy: Teratogenic Effects: Pregnancy Category B. Reproduction studies have been performed in rats, rabbits and mice at doses up to six times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to the drug. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, the drug should not be used during pregnancy unless clearly needed.

Non-teratogenic Effects: In rats, pregnancy was prolonged when the drug was given before the onset of labor; where it was given after the delivery process had begun, labor was protracted.

Nursing Mothers: Caution should be exercised if the drug is administered to a nursing woman since the naproxen anion has been found in the milk of lactating women at a concentration of approximately 1% of that found in the plasma.

Pediatric Use: Pediatric indications and dosage have not been established.

Adverse Reactions: Adverse reactions reported in controlled clinical trials in 960 patients treated for rheumatoid arthritis or osteoarthritis are listed below. In general, these reactions were reported 2 to 10 times more frequently than they were in studies in the 962 patients treated for mild to moderate pain or for dysmenorrhea.

Incidence Greater Than 1%: Gastrointestinal: The most frequent complaints reported related to the gastrointestinal tract. They were: constipation,* heartburn,* abdominal pain,* nausea,* dyspepsia, diarrhea, stomatitis.

Central Nervous System: Headache,* dizziness,* drowsiness,* lightheadedness, vertigo.

Dermatologic: Itching (pruritis),* skin eruptions,* ecchymoses,* sweating, purpura.

Special Senses: Tinnitus,* hearing disturbances, visual disturbances.

Cardiovascular: Edema,* dyspnea,* palpitations.

General: Thirst.

*Incidence of reported reaction between 3% and 9%. Those reactions occurring in less than 3% of the patients are unmarked.

Incidence Less than 1%: Probable Causal Relationship: The following adverse reactions were reported less frequently than 1% during controlled clinical trials and through voluntary reports since marketing. The probability of a causal relationship exists between the drug and these adverse reactions: congestive heart failure, renal disease, glomerular nephritis, interstitial nephritis, nephrotic syndrome, abnormal liver function tests, hematuria, jaundice, thrombocytopenia, leukopenia, granulocytopenia, gastrointestinal bleeding, peptic ulceration with bleeding and/or perforation, hematemesis, melena, vomiting, eosinophilia, pyrexia (chills and fever), skin rashes, menstrual disorders, myalgia and muscle weakness, alopecia, inability to concentrate, depression, malaise, dream abnormalities.

Causal Relationship Unknown: Other reactions have been reported in circumstances in which a causal relationship could not be established. However, in these rarely reported events, the possibility cannot be excluded. Therefore these observations are being listed to serve as alerting information to the physicians: angioneurotic edema, agranulocytosis, aplastic anemia, hemolytic anemia, hypoglycemia, hyperglycemia, urticaria.

Overdosage: Significant overdosage may be characterized by drowsiness, heartburn, indigestion, nausea or vomiting. Because naproxen sodium may be rapidly absorbed, high and early blood levels should be anticipated. No evidence of toxicity or late sequelae have been reported 5 to 15 months after ingestion for three to seven days of doses equivalent to up to 3,300 mg of naproxen sodium. One patient ingested a single dose equivalent to 27.5 g of naproxen sodium and experienced mild nausea and indigestion. It is not known what dose of the drug would be life threatening. The oral LD$_{50}$ of the drug is 543 mg/kg in rats, 1234 mg/kg in mice, 4110 mg/kg in hamsters and greater than 1000 mg/kg in dogs.

Should a patient ingest a large number of tablets, accidentally or purposefully, the stomach may be emptied and usual supportive measures employed. Animal studies suggest that the prompt administration of 5 grams of activated charcoal would tend to reduce markedly the absorption of the drug. It is not known if the drug is dialyzable.

Dosage and Administration: For Mild to Moderate Pain and Dysmenorrhea: The recommended starting dose is two 275 mg tablets, followed by one 275 mg tablet every 6 to 8 hours, as required. The total daily dose should not exceed 5 tablets (1,375 mg)

For Rheumatoid Arthritis and Osteoarthritis: The recommended starting dose in adults is one 275 mg tablet twice daily (morning and evening) or one 275 mg tablet in the morning and two 275 mg tablets in the evening. During long-term administration, the dose may be adjusted up or down depending on the clinical response of the patient. A lower daily dose may suffice for long-term administration. Daily doses higher than 1100 mg in these indications have not been studied. The morning and evening doses do not have to be equal in size and the administration of the drug more frequently than twice daily is not necessary. Symptomatic improvement in arthritis usually begins within two weeks. However, if improvement is not seen within this period, a trial for an additional two weeks should be considered.

How Supplied: ANAPROX® (naproxen sodium) is available in filmcoated tablets of 275 mg (light blue), in bottles of 100 tablets (NDC 18393-274-42) and 500 tablets (NDC 18393-274-62). Store at room temperature in well-closed containers.

SYNTEX
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Endometrial adenocarcinoma arising in adenomyosis

ENRIQUE HERNANDEZ, M.D.
J. DONALD WOODRUFF, M.D.
Baltimore, Maryland

Endometrial adenocarcinoma and adenomyosis are both common problems arising in the uterus. Interestingly, there are few reports of the combination of these two pathologic entities. A recent experience with eight cases found in the files of the Gynecologic Pathology Laboratory at The Johns Hopkins Hospital between the years 1955 and 1974 are reported. Of significance is the differential in prognosis between such cases with deep involvement and true invasive disease. (AM. J. OBSTET. GYNECOL. 138:827, 1980.)

THE CLASSIC prognostic factors employed in the evaluation of endometrial adenocarcinoma have been: (1) the length of the uterine cavity, (2) differentiation of the tumor, and (3) the depth of myometrial invasion. Some investigators feel that the latter is the most significant since penetration through more than 50% of the myometrium has been associated with an increased incidence of lymph node metastases and thereby reduced survival rates regardless of the histopathology or size of the uterus.

Many have appreciated the frequent finding of adenomyosis in routine histopathologic study of any uterus regardless of the indication for the hysterectomy, with incidences varying from 30% to 50%, depending on the number and distribution of the sections.[1, 2] There are also numerous studies identifying the significance of pathologic changes in extrauterine

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Received for publication June 8, 1980.

Accepted August 13, 1980.

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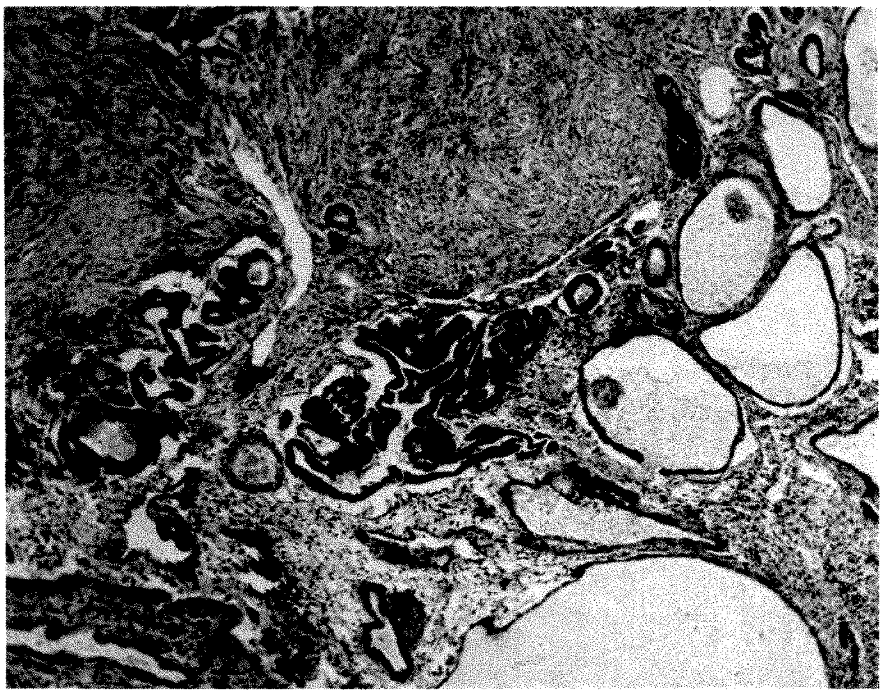

Fig. 1. Typical and atypical hyperplasia in focus of adenomyosis. (×90.)

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Table I. Data on eight cases of endometrial adenocarcinoma originating in adenomyosis

Case No.	*Age (yr)*	*Race*	*Parity*	*Years PM*	*Symptoms*	*Stage*	*Histopathology grade*	*Myometrial invasion*
1	38	W	0	0	IMB	IA	1	Superficial
2	54	B	9-0-0-9	0	IMB	IA	1	Superficial
3	66	B	0-0-6-0	27	PMB	IA	2	Superficial
4	50	W	2-1-0-3	0	MM	IA	1	Superficial
5	80	W	2-0-0-1	29	PMB	IB	2	Superficial
6	62	W	0	2	—	—	1	Superficial
7*	55	—	—	—	—	—	1	Superficial
8*	—	—	—	—	—	—	1	None

PM, Postmenopausal; IMB, intermenstrual bleeding; PMB, postmenopausal bleeding; MM, menometrorrhagia; TAH-BSO, total abdominal hysterectomy and bilateral salpingo-oophorectomy; Irrad., irradiation; NED, no evidence of disease.

*Referred case.

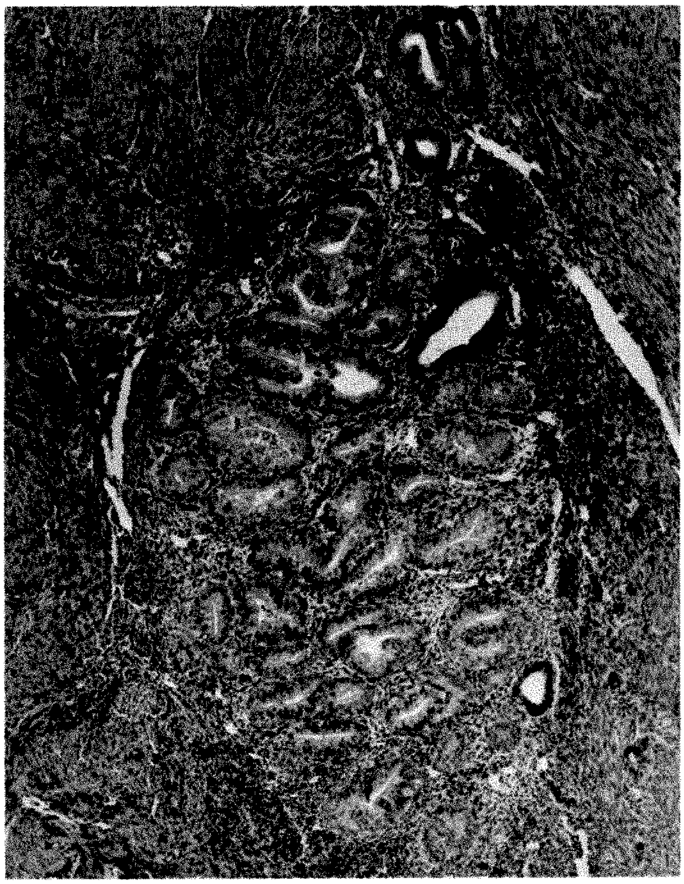

Fig. 2. Atypical hyperplasia in adenomyosis—note transition at arrow. (×75.)

endometriosis.[3-6] In contrast there are few reports which examine the frequency and importance of such alterations in adenomyosis. Furthermore, since most pathologists recognize that intrauterine histopathologic changes, for example, hyperplasia, may be replicated in adenomyotic foci, and since depth of invasion by endometrial adenocarcinoma is of major importance, the differential survival between the patients with myometrial invasion and those with malignancy arising in adenomyosis may be prognostically significant.

In an effort to establish the possible difference in prognosis between the latter, cases of primary endome-

Depth of adenocarcinoma in adenomyosis (%)	*Therapy*	*Follow-up*
25	TAH-BSO	NED, 5 yrs
25	TAH-BSO Irrad.	NED, 5 yrs
>50	TAH-BSO Irrad.	NED, 8 yrs
25	TAH-BSO	NED, 6, yrs
25	TAH-BSO Irrad.	NED, 6 yrs
100	TAH-BSO	NED, 9 yrs
100	TAH-BSO	NED, 10 yrs
75	TAH-BSO	NED, 6 yrs

trial adenocarcinoma were studied for the presence of adenomyosis and the varying degrees of histopathologic alteration found in these adenomyotic foci.

Material and methods

Of 204 patients undergoing hysterectomy for endometrial adenocarcinoma on the gynecologic service of The Johns Hopkins Hospital during the years 1955 to 1974, 21 (10%) were found to have associated adenomyosis. The latter diagnosis was made only when endometrial glands and stroma were found in the myometrium more than one high-power microscopic field below the basal endometrium.

All histologic material from these cases was reviewed and additional sections were cut only if the original slides were considered inadequate.

Clinical data. Clinical data were available on 20 of the 21 cases. The youngest patient was 38 and the oldest was 84 years of age; 15 were white and 5 were black; 16 were multigravid and four were nulligravid.

Symptoms. As might be expected in patients harboring both endometrial cancer and adenomyosis, abnormal uterine bleeding was the presenting complaint in all cases.

Pathology. Review of the sections from 21 uteri with adenomyosis and adenocarcinoma revealed that, in foci of adenomyosis, atypical endometrial hyperplasia was present in two cases and adenocarcinoma was present in six cases. The atypical hyperplasia was characterized by loss of intervening stroma resulting from aggressive epithelial proliferation. Individual cell atypia was not significant (Figs. 1 and 2).

The adenocarcinoma in adenomyosis was well differentiated in all cases. Myometrial involvement was present to a depth of less than 50% in four instances and to more than 50% in two cases.

In addition to the above six cases from the files of The Johns Hopkins Hospital, there were two referred cases in which adenocarcinoma in adenomyosis involved more than 50% of the myometrium, and in one malignant alterations were recognized in adenomyosis adjacent to the serosal surface (Fig. 3).

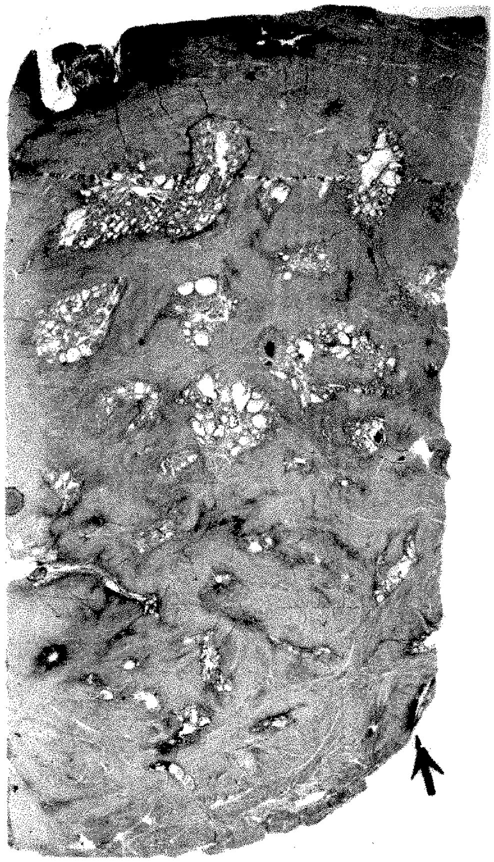

Fig. 3. Diffuse adenomyosis with focal adenocarcinoma—involvement to serosa at arrow. (×35.)

As is true in most cases of well-differentiated carcinoma, individual cell anaplasia was not found to be a significant histopathologic feature and the diagnosis was established primarily by pattern rather than cell detail. Pseudostratification of the epithelium, mitotic activity, and marked adenomatous crowding of the glands with papillary infolding into the lumina were the prominent findings. The differential between direct myometrial invasion and tumor developing in adenomyosis was based primarily on Sampson's criteria as noted below, namely, the demonstration of a direct transition from benign to malignant and the finding of stroma with the epithelial elements (Fig. 4). In contrast, metastatic or invasive foci classically lack the stromal component (Fig. 5).

Treatment and results

All cases were treated with abdominal hysterectomy and bilateral salpingo-oophorectomy. In three of the

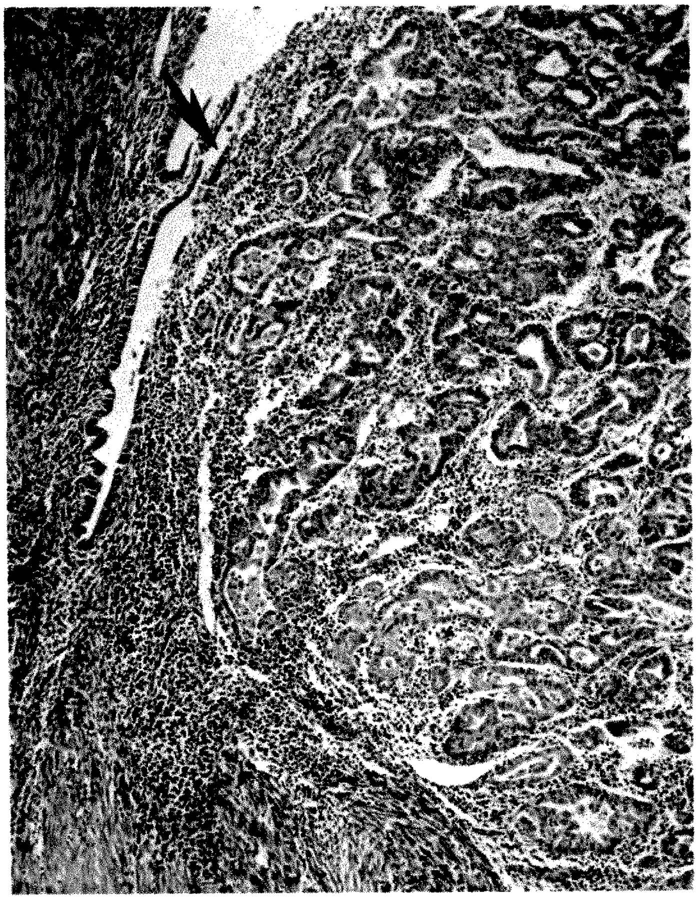

Fig. 4. Adenocarcinoma beginning in adenomyosis. Note transition from benign adenomyosis to cancer at arrow. (×90.)

eight cases adjunctive postoperative pelvic irradiation was given. In the latter, the malignant alteration in adenomyosis was found in the inner 25% of the myometrium in two instances while in the third case the outer half of the myometrium was the site of neoplastic change. In the remaining five cases, the adenocarcinoma in adenomyosis was present in the inner 25% of the myometrium in two instances and at a depth of 75% in one case; in the other cases, the subserosal myometrium was involved. All patients are living and well 5 or more years without evidence of disease (Table I).

Comment

The development of adenocarcinoma in ectopic endometrium has been documented by numerous investigators. In 1925, Sampson[5] established the criteria for the acceptance of such an association in the ovary, and Scott,[6] in his review of the subject in 1953, reaffirmed the significance of these criteria. Briefly they are: (1) benign and malignant tissues coexist in the same ovary; (2) carcinoma *must arise* in a focus of endometriosis, thus eliminating the possibility of metastatic disease; and (3) supporting evidence includes the finding of tissue resembling endometrial stroma surrounding characteristic glands.

Scully and associates[3] reported 17 cases of endometrioid carcinoma in the ovary, four of which fulfilled Sampson's criteria. These authors confirmed the opinion of previous investigators that the occurrence of such a sequence of events as described by Sampson was uncommon. Brooks and Wheeler[4] reviewed the literature relative to malignancy arising in extragonadal endometriosis and accumulated 46 cases from the literature including one of their own.

In spite of these many references to carcinoma arising in endometriosis at extrauterine sites and the well-known frequency of endometrial adenocarcinoma, there are few documented cases of neoplasia developing in adenomyosis with or without associated intrauterine cancer. Rolly[7] is given credit for the initial report in 1897, and one such case is recorded in Cullen's[8] monograph on adenomyoma published in 1908. Although Borowski[9] collected 26 cases in 1929, only two including his own can be considered well-docu-

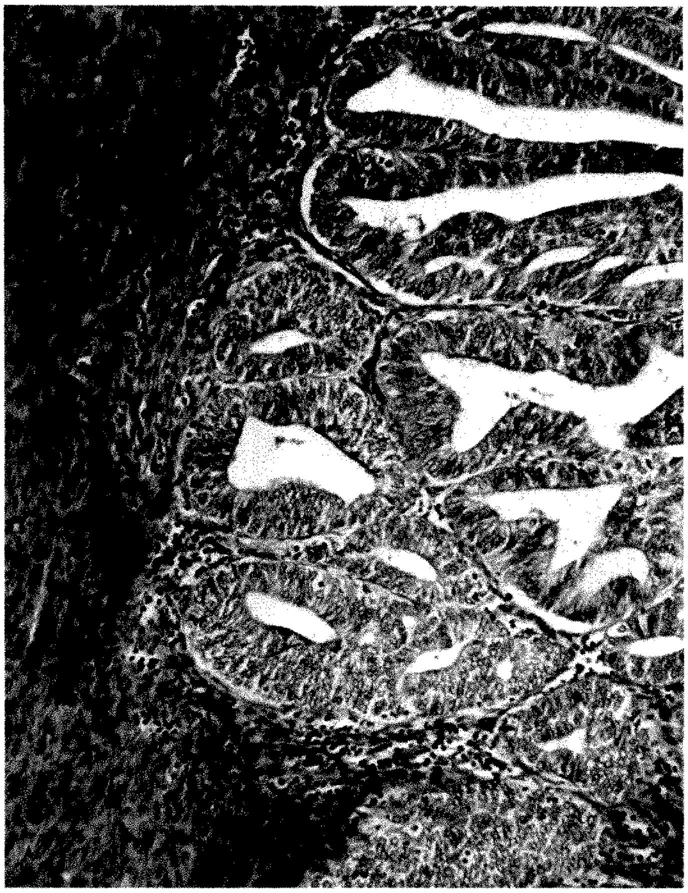

Fig. 5. Invasive adenocarcinoma—direct invasion without a stromal component. (× 130.)

mented demonstrations of adenocarcinoma in adenomyosis, since the majority of the lesions were apparent examples of adjacent malignancy invading adenomyosis.

Review of the literature revealed 16 documented instances of adenocarcinoma in adenomyosis. In eight cases the malignancy apparently arose at both the intrauterine and adenomyotic sites.[9-14] In three of Colman and Rosenthal's[10] four cases of endometrial adenocarcinoma, there was evidence of "carcinoma within areas of adenomyosis"; however, it is difficult to determine whether this was direct invasion or representative of separate and distant alterations. In the remaining eight cases, the neoplasia apparently developed in adenomyosis without adenocarcinoma in the intrauterine endometrium (Table II).[7, 8, 11, 15-19]

In the present series adenomyosis was found in association with endometrial adenocarcinoma in 10% of 204 cases. Others[20-23] have reported incidences of such association in 16%, 19.4%, 33%, and 60%; however, malignancy arising in the adenomyotic foci was not discussed although "considered" by Marcus.[23]

In the current series of 21 cases in which there was an association of adenocarcinoma and adenomyosis, six (30%) demonstrated malignant alteration in both surface and intramyometrial endometrium. An additional two cases referred to the laboratory are included in the final analysis since careful histopathologic study and clinical evaluation have been possible in both instances.

Table II. Literature review: Cases of adenocarcinoma in adenomyosis

Authors	*Without intrauterine malignancy*	*With intrauterine malignancy*
Andrews[15]	1 (?)	—
Baril and associates[19]	1	—
Borowski[9]	—	1
Colman and Rosenthal[10]	—	3 (?)
Cullen[8]	1 (?)	—
Dockerty[11]	1	1
Dubrausky and Niendorf[16]	1 (?)	—
Eisner[17]	1 (?)	—
Grayzel[12]	—	1
Greene[18]	1	—
Kumar and Anderson[13]	—	1
Rolly[7]	1 (?)	1
Winkelman and Robinson[14]		

This remarkably high association of malignancy at separate sites, in both the intrauterine and adenomyotic endometrium, compared with the few reported cases, can only be attributed to a more careful evaluation of the histopathologic material or differences in the interpretation of the myometrial disease. We feel that the latter is the more realistic interpretation. Of major significance, however, is the differential in survival between deeply invading adenocarcinoma and that arising in separate foci. This difference has been elaborated in the study of cases of endometrial and ovarian neoplasia in which the lesions developed at separate sites as contrasted with true metastatic disease.[24, 25]

Although this series is small, it represents an approach to differential diagnosis between metastatic and multifocal disease. Certainly more studies are needed in order to confirm or refute the opinion that there is a remarkable differential in prognosis between these conditions.

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Tritiated water exchange in the human uterine cavity

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Measurements with the use of tritiated water (^3H_2O) in human volunteers indicate that the mean tritiated water half life (t½ ^3H_2O) in the uterine cavity is 1.3 minutes. This figure is very close to that previously measured in subhuman primates.[1] By extrapolation from various prior studies, the volume of the human uterine cavity (aV_c) may be assumed to approximate 0.4 to 0.6 ml. According to the formula $\frac{aV_c}{2} \times \frac{1,440}{(t½\ ^3H_2O)}$ water turnover in the human endometrial canal is calculated to range from 222 to 332 ml/24 hours. (AM. J. OBSTET. GYNECOL. 138:833, 1980.)

DATA ON UTERINE CAVITY water turnover in humans should be useful in devising intrauterine medication delivery systems. If the half life (t½) of a given medication in uterine cavity water and the cavity water turnover rate are known, the delivery rate from an intrauterine device necessary to maintain a given concentration inside the uterine lumen can be calculated. Medication half lives can be determined from radiolabeled medications deposited in the endometrial canal. Uterine cavity volume and water t½ must be determined in order to calculate water turnover in the uterine canal.

Few studies bearing on uterine luminal volume and fluid turnover in mammals have been performed. Previous investigations on human uterine cavity size have determined only dimensions of length and width and characterized its general shape. These studies have been performed: (1) by casting the cavity of surgical or autopsy specimens with various substances,[2,3] (2) by examining hysterosalpingograms,[4,5] and, most recently, (3) by using a "uterine metrology device."[6] Attempts at measuring endometrial fluid production in rats and mice have been made by closure of the lower uterine horns or cervices and, after a period of time, aspiration of accumulated fluid.[7,8] Volume of fluid production has also been attempted in ewes by collection from an indwelling catheter with the lower end of the uterine horn ligated.[9]

In an effort to assess water volume and turnover rate in the uterine cavity (endometrial canal) under physiologic circumstances, Shaw and associates[1] measured the tritiated water (^3H_2O) volume and exchange in uterine cavities of monkeys. This paper reports on similar measurements carried out in human volunteers.

Material and methods

The materials and instruments used were the same as previously described,[1] with one exception. For infusion of the ^3H_2O solution into and for washout of the human uterine cavity through the cervical canal, a modified Lippes Loop inserter with a prethreaded fine Teflon tubing was used (Fig. 1).

The methods were based on the following principles and assumptions used previously in experiments with rhesus and cynomolgus monkeys[1]: (1) If the uterine cavity fluid is replaced by a physiologic solution containing ^3H_2O, the radioactive water in the cavity will be diluted in time by exchange with that water which continually enters and leaves the endometrial canal by processes of diffusion and secretion, absorption, and flow through the cervical canal and possibly the fallopian tubes. If the amount of tritiated water remaining in the cavity is determined at varying time intervals after the endometrial fluid is originally replaced with the ^3H_2O solution, the t½ ^3H_2O can be determined graphically from the negative curve of the remaining ^3H_2O in the cavity versus time (Fig. 2). (2) By extrapolation of the

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Supported by a grant from Alza Research, A Division of Alza Corporation, Palo Alto, California.

Received for publication June 9, 1980.

Accepted August 13, 1980.

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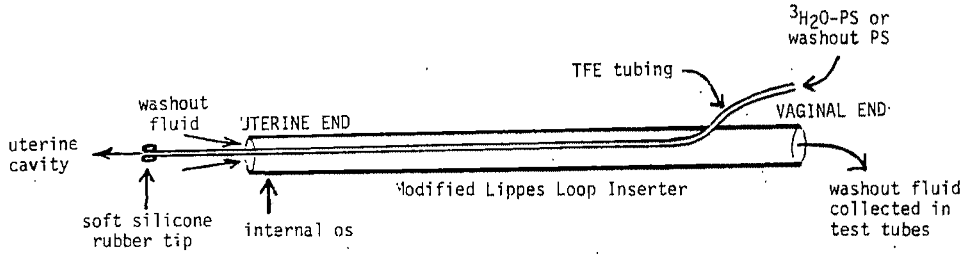

Fig. 1. This demonstrates a plastic Lippes Loop inserter into which a hole is drilled near the vaginal end to snugly accomodate Teflon *(TFE)* tubing (0.38 mm inside diameter) tipped by atraumatic silicone rubber tubing (0.94 mm outside diameter). ^3H_2O in PS is infused or injected into the uterine cavity through the Teflon tubing. At intervals after injection, the remaining ^3H_2O in the endometrial canal is flushed out by infusing 15 ml of PS at 3 ml/min. The washout fluid is collected from the vaginal end of the inserter into serial fractions.

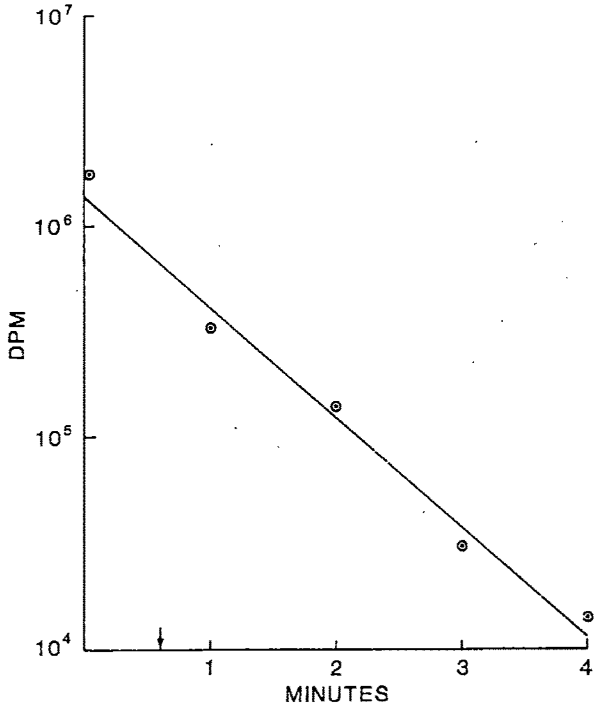

Fig. 2. Curve of ^3H_2O disappearance from uterine cavity with time in one experiment on Subject 3. Arrow is at t½ ^3H_2O = 0.58 minutes.

negative curve to zero time (t = 0), the amount of ^3H_2O in the cavity initially may be derived. Assuming the cavity volume to be of physiologic dimensions at t = 0, the amount of ^3H_2O in the cavity at t = 0 divided by the specific radioactivity of the ^3H_2O solution infused equals the volume of distribution of cavity water, or the apparent uterine cavity volume (aV_c). Thus, daily water turnover in the uterine lumen may be calculated from the equation $\frac{aV_c}{2} \times \frac{1,440}{t½\ ^3H_2O}$.[1]

Thirty-five satisfactory experiments were carried out on four female volunteers. All four women were determined to be in good general health by history and physical examination. They all had regular, subjectively normal menstrual periods varying from 28 to 32 days in length, and none of the women was using an intrauterine contraceptive device or hormonal contraception. None of the women had been pregnant within the prior year. Ovulatory cycles were confirmed in all by serum progesterone levels exceeding 4 ng/ml on days 21 to 23 following the first day of the last menstrual period. Other data are shown in Table I. Menstrual diary cards were used so that accurate times in the menstrual cycle could be recorded when experiments were performed. All subjects signed informed consents detailing all possible risks which could be anticipated. The women were instructed to come to the clinic for measurement on days 6, 11, 16, 21, and 26 of the cycle. No measurements were made during menstruation.

Experiments were performed with the subjects in the lithotomy position. With the use of a sterile technique, the modified Lippes Loop inserter with its prethreaded Teflon tubing was put into the cervical canal and the uterine end was extended to the level of the internal os (Fig. 1). The endocervical canal was often dilated gently with a series of uterine sounds in order to allow the modified inserter to completely fill the canal at the internal os without undue stretching. This dilation and insertion never resulted in the appearance of blood at the external os or in the infusion or washout fluid. The cavity end of the Teflon tubing, initially retracted inside the inserter, was gently threaded from the level of the internal cervical os to the uterine fundus and then withdrawn 2 to 4 mm. One of two procedures was then carried out. The uterine cavity was either infused with 15 ml of ^3H_2O in physiologic solution (PS) or injected with 10 μl of ^3H_2O in PS. In the latter case, the distal portion of the Teflon tubing was filled prior to inser-

Table I. Data on four female volunteers

Subject	*Age*	*Obstetric history**	*Uterine sound depth (cm)*	*Other data*
1	29	3/3/0	7.5	Laparoscopic tubal ligation 1 year previously
2	21	1/1/0	7.5	No contraception
3	43	5/3/2	7.5	Abdominal tubal ligation 15 years earlier
4	27	3/3/0	8.0	No contraception (husband underwent vasectomy)

*Gravidity/parity/abortions.

tion with 10 μl of ^3H_2O in PS by means of a calibrated microliter syringe. In both cases, the specific radioactivity of the infusion or injection fluid was known and usually approximated 1×10^8 dpm/ml. The infusion fluids were warmed to 40° C before flushing. The composition of the PS, which had a pH of 7.84 and an osmolality of 271 mOsm/kg, was the same as that previously used in monkeys.[1] Whenever either ^3H_2O in PS or washout PS was injected or infused, the Teflon tubing was gradually withdrawn into the inserter during the last portion of the injection or infusion. After the infusion or injection was stopped, the inserter was withdrawn. Any solution which spilled into the vagina during infusion or withdrawal of the inserter was absorbed into a cotton sponge and discarded. The rate of all infusions was 3 ml/minute and was controlled by a constant-infusion pump.

After usual intervals of 1.0, 2.0, and 3.0 minutes following infusion or injection of ^3H_2O in PS, the endometrial canal was infused with 15 ml of PS to wash out the remaining ^3H_2O. The washout fluid, issuing from the vaginal end of the the inserter, was collected serially in fractions of approximately 1 ml. The volume of fluid collected in each tube was determined by weighing the tubes before and after collection. In this way the exact volume was calculated and, when multiplied by the count rate subsequently determined in a liquid-scintillation counter, the total ^3H_2O per fraction was determined.

In each satisfactory experiment, three or more tritiated water injections followed by an equal number of washout infusions were performed in order to determine the amount of ^3H_2O remaining at approximately 1.0, 2.0, and 3.0 minutes after injection. In the majority of these experiments, one or more intervals were duplicated to determine the degree of variation in repeated measurments. In all other ways, the methods and calculations were the same as those previously described for measurements in subhuman primates.[1]

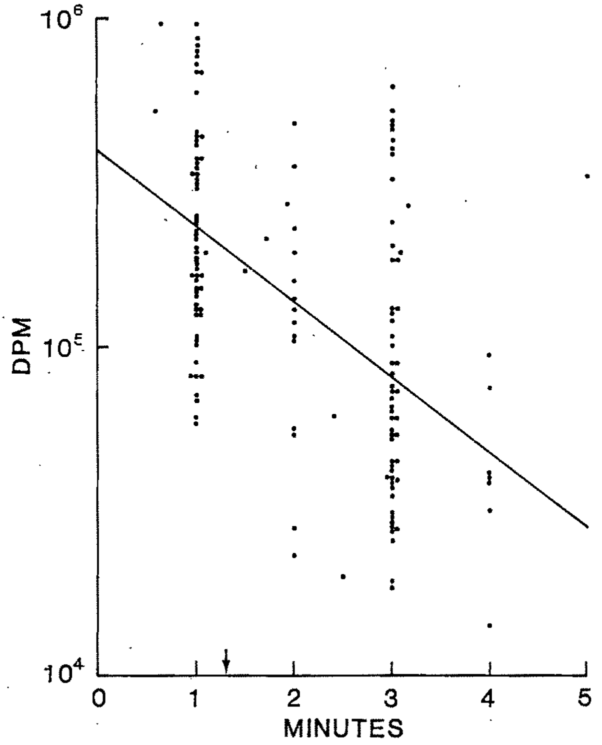

Fig. 3. Composite regression curve of ^3H_2O disappearance from uterine cavity with time. The graph includes 144 data points from 35 experiments on four subjects. The correlation coefficient for these data is −0.53 ($p < 0.01$). The arrow points to t½ ^3H_2O = 1.32 minutes derived from the curve.

Results

Initially in the study, 15 ml of ^3H_2O in PS was infused into the uterine lumen in order to replace endometrial fluid with ^3H_2O in PS at $t = 0$. This is necessary for the determination of aV_c. A series of unsatisfactory experiments followed. In contrast to prior experiments on monkeys, after two or more infusions of ^3H_2O in PS and PS washout cycles, recovery of infusion fluid volume through the modified Lippes Loop inserter rapidly declined. This suggested that repeated infusions may have been associated with an opening of the proximal oviducts and loss of infused fluid into the fallopian tubes. In fact, experiments on one woman (Subject 2) repeatedly resulted in poor returns of infused volume whenever the procedure was carried out on or near day 16 of the cycle. An experiment was not considered satisfactory unless all infusions were associated with recoveries ≥95% of the infused volume.

In order to decrease the number of infusions required per experiment and still to measure at least three time intervals after ^3H_2O instillation, a 10 μl injection of ^3H_2O in PS was used instead of the 15 ml infusion of ^3H_2O in PS. In this way, we were able to measure two to three intervals in each subject during

Table II. Data on ^3H_2O exchange rates in four women

Subject No.	*Range of t½ ^3H_2O in minutes*	*Mean t½ ^3H_2O in minutes*	*No. of experiments*	*Range of correlation coefficients*
1	0.61-0.90	0.76	2 (9*)	−0.84 to −0.98
2	0.76-3.20	1.56	5 (20)	−0.89 to −1.00
3	0.42-0.81	0.64	4 (19)	−0.86 to −1.00
4	0.92-2.56	1.68	6 (25)	−0.86 to −1.00
Total	0.42-3.20	1.29	17	−0.95 (mean)

*No. of data points.

Table III. Mean ^3H_2O exchange rates during three phases of the ovarian cycle

Subject No.	*No. of experiments*	*Mean t½ ^3H_2O (minutes)*		
		Follicular	*Periovulatory*	*Luteal*
1	2		0.63	0.91
2	7	1.64*	1.30	1.12
3	4	0.72		0.73
4	7	1.96†	1.50	1.39
Grand mean		1.44	1.14	1.04

*Includes one very high result of 3.20 minutes.

†Includes one high result of 2.56 minutes.

35 satisfactory experiments. As many as six successful measurements could be carried out in several experiments. Thus, many time intervals were repeated at least once in the majority of experiments, and the variation was calculated. The percent difference was calculated for 34 intervals which were repeated during the same experiment. These differences ranged from 1% to 13% and averaged 3.6%. In most cases the 1.0- and 3.0-minute intervals were repeated nonconsecutively. An additional advantage of injecting a known amount of radioactivity at $t = 0$ was that it provided an additional data point for each experiment, that is, disintegrations per minute in the uterine canal at $t = 0$ (Fig. 2).

Fig. 3 demonstrates the individual data (144 points) from satisfactory experiments in four volunteers. The regression curve from this composite data indicates a mean t½ ^3H_2O in the human uterine cavity of 1.32 minutes. The correlation coefficient determined for these data was −0.53, which is associated with a $p < 0.01$ that the data coordinates were randomly distributed.

Table II summarizes the data of the 17 best individual experiments, i.e., those associated with correlation coefficients of −0.84 to −1.00. The mean t½ ^3H_2O was 1.29 minutes, a figure in close agreement with the t½ derived from the composite data. Individual t½ ^3H_2O measurements ranged from 0.42 to 3.20 minutes.

Table II demonstrates an eightfold variation of the water exchange rate (t½) among the four subjects studied at various times, and for one subject there was a greater than fourfold variation among individual experiments (Subject 2). Since these subjects had very regular cycles and kept diary cards, it was possible to estimate the degree of variation among all subjects during the ovarian cycles. For purposes of statistical analysis, the ovarian cycle of each volunteer was divided into three phases: follicular, periovulatory, and luteal. The earliest measurements were made on day 6 and the latest, on day 26; thus, for purposes of analysis, days 6 to 11 were considered follicular; days 12 to 18, periovulatory; and days 19 to 26, luteal.

When composite data were used, the t½ ^3H_2O during the follicular phase was found to be 1.20 minutes, that for the periovular phase was 1.48 minutes, and that for the luteal phase was 1.16 minutes. Table III demonstrates the mean t½ ^3H_2O measured in 20 experiments on the four subjects. No significant difference exists for $\alpha = 0.05$ in the Kruskal-Wallis nonparametric one-way analysis of variance.[10] The composite data indicated that the periovulatory phase is associated with the longest t½, whereas the selected data shown in Table III indicated that the follicular phase is associated with a longer t½. However, neither analysis indicated a statistically significant difference between phases.

Experiments with the use of ^3H_2O indicate that the uterine cavity of the human exchanges half its contained volume of water in approximately 1.3 minutes on the average (t½ $^3H_2O \equiv 1.3$ minutes). In experiments performed in vivo on four women volunteers, we measured t½ ^3H_2O varying from 0.42 to 3.20 minutes. This variation did not appear to depend significantly on the phases of the ovarian cycle. However, these observations relied on a relatively small number of experiments performed during the menstrual cycles of only four women. More extensive studies may reveal statistically significant differences in various periods of the ovarian cycle. That a dramatic difference did not regularly occur between various phases at first seems surprising. However, the exchange rate of water may be much less affected during the monthly changes of the uterine endometrium than are the exchange rates of other substances in the endometrial fluid, particularly proteins and mucoproteins.

Previous experiments with radioisotopic water have resulted in measurements of apparent uterine cavity volume, t½ ^3H_2O, and water turnover in subhuman primate models.[1] By means of similar technique reported here, we were able to estimate t½ ^3H_2O but not the apparent uterine cavity volume or water turnover in female human volunteers. However, by extrapolation from our previous work on primates and various published experiments in which uterine cavity size was determined in humans, an estimation can be made of cavity volume and, in conjunction with measured t½ ^3H_2O, human uterine lumen water turnover. Various measurements of uterine cavity dimensions are consistent with a volume of 0.600 ml.[1-6] Apparent uterine cavity volume measured in monkeys was 0.032 ml.[1] Since women of reproductive age are approximately 13 times heavier than rhesus monkeys of reproductive age,[11] aV_c could be assumed to be approximately 13 times greater in humans, or 0.416 ml. Thus, several types of experimentation suggest that the mean volume of the human endometrial canal is in the range of 0.400 to 0.600 ml. With the assumption that this is true and that t½ ^3H_2O is 1.3 minutes, water turnover in the human uterine cavity would be (from equation 1)

$$\frac{0.4\text{-}0.6}{2} \times \frac{1{,}440}{1.3} = 222 \text{ to } 332 \text{ ml/24 hours.}$$

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CORRESPONDENCE

Laser therapy of cervical intraepithelial neoplasia

To the Editors:

In the article by Baggish, entitled "High-power-density carbon dioxide laser therapy for early cervical neoplasia," which appeared in the January 1, 1980, edition of the GRAY JOURNAL, on page 117, Dr. Baggish claims a high cure rate with high-power-density carbon dioxide laser therapy for cervical intraepithelial neoplasia (CIN). The primary failure rate with laser in Dr. Baggish's hands is 26% and it is 32% for CIN III. For cryosurgery, the primary failure rate is around 10%.[1] The failure rate after second therapy, in Dr. Baggish's hands, was approximately 7%, which is no better than that with cryosurgery. Baggish criticizes cryosurgery because it supposedly has major hazards and moves the squamocolumnar junction into the canal. Where are his data?

When compared to conization, which has a failure rate of less than 4%, according to an article by Kolstad which Dr. Baggish cited, laser rates very poorly. The results obtained by Carter and Stafl and their associates, who used low-power density, both of whom he cited, had a lower primary failure rate than that of Dr. Baggish. Their overall cure rate was lower than his, after two treatment sessions.

The treatment of diethylstilbestrol (DES)-related epithelial changes is totally unnecessary and represents meddlesome medicine.

The 15 cases of carcinoma in situ in DES-exposed offspring in his 120 patients is three times as many as we have in the 4,000 DES-exposed offspring in the nationwide National Cooperative Diethylstilbestrol Adenosis Project. Perhaps not all of his patients had a legitimate carcinoma in situ.

The use of electrodiathermy by Chanen and Hollyock,[2] which requires general anesthesia, has a superb cure rate with very few complications. I know of no single death in Australia, and they have treated more than 2,000 patients in Sidney and Melbourne.

The recommendations for caution must be strongly emphasized since more carefully controlled studies must be carried out with the laser before it can be considered a legitimate treatment modality.

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Reply to Dr. Townsend

To the Editors:

Dr. Townsend's letter unfortunately extracts data from my article, "High-power-density carbon dioxide laser therapy for early cervical neoplasia," out of context. He quite surely knows that *all* cases treated by laser were included in these statistics, i.e., even patients with early CIN who were treated to a shallow depth by laser beam prior to the current routine removal of the entire squamocolumnar junction down to 5 mm. Retreatment was done at 3 weeks and again at 6 weeks. Since the first 50 patients were examined every other day and since early follow-up therapy was very aggressive, many of these "retreatments" were most probably carried out for atypical metaplasia, a finding prevalent in the healing phase. Primary therapy in this study was considered to terminate after 6 weeks, i.e., after 6 weeks patients were examined every 6 months. In actuality this compares favorably with cryosurgery standards where in fact every patient initially receives two treatments. Complete healing had occurred in the cases requiring two additional laser exposures before the cervical discharge had stopped in a like group of cryosurgery-treated patients. These criteria are in fact no different from those suggested in an article which Townsend coauthored: "Two cryosurgical treatments were used in most patients before it was considered a failure."[1] The long-term cure rate in my series was 95%, i.e., there were five failures in 115 cases.

Dr. Townsend cites a cryosurgical failure rate of 10% but supplies no follow-up data. Again in the article he coauthored one year later, the overall residual failure rate was 19% in the entire group of 118 cases and 33% in the CIN III group. The latter cryosurgical failure statistics are corroborated in several other published series. Creasman and associates [2] reported persistent CIN following cryotherapy in 22 of 75 patients (30%). When a single treatment was given, the failure rate was 48% as compared to 18.7% when two treatments were performed. Gray and Christopherson[3] routinely treated 66 women twice while other patients had even more freezings. Sixteen patients had persistence of dysplasia or carcinoma in situ (a failure rate of 24%). Most recently, Walton and associates[4] reported failure rates of 24.2% (moderate dysplasia) and 31.6% (severe dysplasia).

A major advantage of laser therapy is that it is truly a microsurgical technique and since minimal necrotic tissue is left behind healing proceeds rapidly with virtually no scar formation. Cryosurgery may sometimes result in severe healing difficulties. In the study of Walton and associates[4] four patients developed cervical stenosis; one patient had acute cervical necrosis, and one experienced severe dysmenorrhea. Dr. Townsend questioned data about severe hazards associated with cryosurgery. There is documented evidence that a defective "O" ring on the cryosurgical probe has led to massive insufflation of carbon dioxide, resulting in carbon dioxide embolus, cardiac arrest, and stroke. Similarly cryosurgery for extensive CIN has led to massive cervical destruction with acute necrosis and sloughing of the entire cervix.

Dr. Townsend next compares results with sharp conization to my laser statistics. Silbar and Woodruff,[5] reviewing biopsy, cone, and finally hysterectomy specimens, reported a 21% rate of residual in situ neoplasia (in the uterus) following sharp conization. Garcia and associates[6] studied 115 patients with carcinoma in situ who had sharp conization and were subsequently treated by total hysterectomy (within a maximum of 6 weeks). Significant residual neoplasia was found in the uterus in 51% of these patients. Within the body of this paper was a table citing 12 references (1957 to 1974) with a rate of residual carcinoma after conization ranging from 16.4% to 50%. Additionally the sequelae after sharp conization may be unpleasant, especially for the young woman. Lee[7] reported on the reproductive outcome of 97 patients following sharp cone biopsy. The spontaneous abortion rate was 27.1% (double the usual quoted incidence). The cesarean section rate was 22.1% and three of the 17 cesarean section patients had a fibrotic cervix which failed to dilate. Cervical incompetence was a contributory factor to 11 labors, resulting in a 14.5% incidence of premature deliveries.

Stafl and associates[8] and Carter and associates[9] reported cure rates that were lower than mine because of lower-power density (and in the former series superficial destruction).

We found 15 in situ neoplasias of the cervix and vagina in 400 cases of DES-exposed women. This does not appear to be an excessive number since most of these cases were referred patients. We most probably saw the patients with the most worrisome lesions whereas the general gynecologist would tend to observe and not refer other DES-exposed patients. All biopsy sections were read by two pathologists and overreading was avoided by not specifically labeling these cases as DES treated but rather including them with other (non-DES) in situ neoplasms.

Finally and most perversely, Dr. Townsend espouses electrodiathermy with general anesthesia in preference to laser beam therapy. Here most certainly is demonstrated the greatest contrast between a precise, exact, microsurgical procedure and gross, unselective mass tissue destruction. The odds favoring a mortality with the required general anesthesia in fact exceed the mortality of the disease under therapy, a paradox inconsistent with logical therapeutic objectives. Phillips[10] found that four of every 10,000 Baltimore residents operated upon died from anesthesia per se (0.04%). In this study, including data from 20 Baltimore hospitals, the mean death rate attributable to anesthesia for female subjects was approximately 3 per 10,000. In 1970, a special committee (New South Wales, Australia)[11] identified 1,215 errors in 286 deaths related to general anesthesia. Chanen and Hollyock[12] also reported five major secondary hemorrhages requiring transfusion and three patients who developed cervical stenosis. I am certain Dr. Townsend is aware that the risk of hepatitis may be as high as 1% per transfusion, with an equally high incidence of the development of chronic liver disease. Further hemolytic and febrile reactions may accompany 3% of all transfusions. These complications are a high price for a woman to pay for treatment of a premalignant lesion when other modalities offering fewer and less severe side effects are available.

Finally, I do not see the laser differing greatly from the scalpel. The energy for delivery of the incision differs but the end result most definitely will depend on the skill of the operator. The laser is not magical; it is simply a fine, precisely controlled surgical instrument.

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Betamethasone in prevention of respiratory distress syndrome

To the Editors:

The recent article by Doran and associates, entitled "Results of a double-blind controlled study on the use of betamethasone in the prevention of respiratory distress syndrome" (AM. J. OBSTET. GYNECOL. 136: 313, 1980), contains so many statistical and analytical deficiencies that some detailed analysis of its content seems justified. The purpose of the article, as stated in the title, was to report the results of a double-blind controlled study on the use of betamethasone in the prevention of respiratory distress syndrome (RDS). The data have been handled in such an uncritical way the casual reader might be seduced into reaching the same conclusions the authors proposed, that is, that the data demonstrate some implied benefit to the administration of betamethasone, at least in terms of prevention of neonatal mortality. The central factors in this paper which need to be considered center around the information contained in Table X. It is clearly demonstrated that when the population of patients is studied by gestational age, there is no difference between the drug and control groups in the incidence of RDS. That table alone deals adequately with the proposed effect of steroid administration with regard to the development of respiratory distress and associated complications in the study population. With that particular table, the paper could and should have concluded without further attempt to deal with the data in any more elaborate fashion.

Several factors were almost completely ignored when the authors listed the perinatal deaths in the drug and control populations as outlined in Table V. It should be noted that among the five deaths in the steroid-treated group, four infants were either stillborn or died of causes unrelated to pulmonary maturity or steroid administration. The only remaining death was in a 25-week, 750 gram infant who in many nurseries might be considered previable. It was also not stated whether or not this child suffered from respiratory distress.

More important, in the "placebo" group the first seven infants were either stillborn or presumably previable by gestational age. Of the remaining seven, one was stillborn, one had a congenital syndrome incompatible with life, and two died of causes presumed to be unrelated to respiratory distress because they were not said to have had RDS. One makes this assumption because the remaining three were said to have had RDS. By the most charitable analysis, then, only three of this entire group died of a disease which steroid administration is designed to prevent. Also, if one proposes to draw the conclusion from this study that betamethasone in some way is associated with increased neonatal survival, one must eliminate from the analysis and subsequent statistics those patients without the potential for survival. As stated above, this results in several denominator difficulties within the subsequent statistical analysis. If one removes all of the stillbirths and predestined neonatal deaths from unavoidable causes, one reduces the treated group to 76 (removing one stillbirth and three babies dying of nonrespiratory causes) and the control group to 52 (removing three stillbirths and five other infants who were previable by weight and/or gestational age). Even if those live-born babies in each group with no presumed viability potential are included, the neonatal mortality rate in the treated group is 4/79 versus that in the placebo group of 8/57. The difference is not statistically significant.

If one evaluates the data included in Table VI, it can be seen that the gestational age at delivery of the placebo group is significantly less than that of the treated group at delivery, although the significance is borderline. A statistical assessment of the differences in birth weights was not provided, which also casts some doubt on the comparability of the two groups. This may reflect upon the difference in incidence of RDS between the two groups which, by the authors' incorrect and uncorrected statistics, is said to be significantly different. The authors pointed out that by gestational age this difference is not significant; therefore, the original statement of reference is meaningless.

Further, the comment regarding the perinatal mortality rate in the two groups is also meaningless, in that it was already acknowledged that the two groups were not comparable. The frequency of stillbirths in the control group as well as the numbers of patients with unavoidable deaths was noticeably higher.

The authors stated: "the neonatal mortality rate was significantly less in the treated group (4/80 or 5%) than in the control group (11/60 or 18%). This difference in neonatal mortality rate was probably due to the statistically significant reduction in the incidence of RDS in the entire treated group (4/80 or 5%) as compared to the control group (10/60 or 17%)." Since the authors had already ignored the fact that the groups were not comparable in overall distribution of gestational age and had already specified that the incidence of RDS when viewed by gestational age does not show significant differences, this conclusion is unjustified. The fallacy of this conclusion becomes more evident when one realizes that the expected outcomes have not been adjusted for unavoidably fatal occurrences in both groups as a result of previable fetuses and anomalies incompatible with life.

The initial intent of the study was admirable. Unfortunately, the data processing and conclusions drawn from this study clearly lead to the conclusion that the study has proved nothing when the data are properly analyzed. One often hears of the futility of comparing apples and oranges. This study, in its published form, is patently a fruit cocktail.

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Reply to Dr. Perkins

To the Editors:

Dr. Perkins, in his criticism of our paper, has focused his attention on a number of areas which I and my colleagues feel require a point-by-point response. The main finding of our paper was that in this double-blind study of the effects of betamethasone in the prevention of RDS there was a statistically significant reduction ($p < 0.05$) in the incidence of RDS when the control group (10/60 or 17%) and the treated group (4/80 or 5%) were compared. There were 60 live births in the placebo group (including one set of twins) and 80 live births in the treated group (including six sets of twins). The numerators and denominators in each of these two fractions refer only to live births (Table VI).

When the drug and placebo groups were broken down into the two main gestational groups (24 to 32 weeks, Table VII) and (33 to 34 weeks, Table VIII), there was still a difference in the incidence of RDS between drug and placebo in each group but it was not statistically significant. Similarly, when we further subdivided the groups into smaller gestational age categories of 4 weeks (Table X), the difference in incidence of RDS was present between drug and placebo groups up to 36 weeks but, as one might expect, only when both drug and placebo groups were totaled was the difference statistically significant. This is obviously a function of the small number of cases in each subcategory. This does not in our opinion detract from the main finding of the study derived from analyzing the incidence of RDS in drug and placebo in all live births which is summarized in Tables VII, VIII, and IX, in that the word "total" at the top of each column refers to live births in each table. This can easily be deduced by looking at the numbers to the left of the total column in each case.

Dr. Perkins' second major criticism is the comparability of the drug and placebo groups, which is summarized in Table VI. First, the p value column indicates a value *only* where there is a statistically significant difference and we apologize for this heading which should have read "value if $p < 0.05$." When we compared the characteristics of the two groups, the birth weight and Dubowitz score (postnatal criteria) of both groups were not different although there was a difference of 1.8 weeks' mean gestational age at delivery (obstetric criteria) between drug and placebo groups. Thus, two of three parameters of fetal maturity did in fact indicate comparability between the two groups.

A third major objection of Dr. Perkins is the question of perinatal mortality. We deliberately listed all stillbirths and neonatal deaths (Table V) and did not "correct" the data for malformations and other causes of perinatal mortality. This decision was made because we felt that "correcting" for certain causes of perinatal mortality could in fact introduce inaccuracies caused by the correction itself. How do we, or any other investigators know for certain what the overall effects of betamethasone are on perinatal mortality? In fact, as pointed out in the discussion, it is quite possible or probable that deaths caused by intraventricular hemorrhage and extreme prematurity may be reduced by betamethasone therapy. How does anyone know what effect betamethasone has in utero and, therefore, how can one exclude stillbirths from the calculations of perinatal mortality? The concept of an unavoidable stillbirth or neonatal death is really acceptable only in a minority of situations in modern perinatal medicine. Thus, all cases were left in for analysis. The result was a statistically significant difference ($p < 0.01$) in perinatal mortality between drug (5/81, 6%) and control (14/63, 22%) groups.

In his third paragraph, Dr. Perkins goes further in his correction process by removing stillbirths and neonates considered "previable" from both groups in his calculation of "corrected" neonatal mortality. We had *already* removed the stillbirths when we calculated neonatal mortality. Even if we subtract from the neonatal deaths the miscellaneous patients suggested by Dr. Perkins (three babies in the treated group dying from nonrespiratory causes and five in the placebo group who were previable by weight and/or gestational age), the comparison becomes 1/77 in the drug group and 6/55 in the placebo group, the difference being significant ($p < 0.05$). Our neonatal mortality was 4/80 or 5% in the drug group and 11/60 or 18% in the placebo group, which is statistically different ($p < 0.05$). As far as we can tell, Dr. Perkins' calculations for neonatal mortality without "corrections" are based on subtracting stillbirths *twice*.

Since we recorded the crude data, the readers including Dr. Perkins are at liberty to draw their own conclusions based on their own interpretations, provided the facts are correctly interpreted. We believe our interpretation is valid though not necessarily exclusive. Finally, Dr. Perkins' comments about "fruit cocktails, etc." are, we feel, inappropriate in the criticism of a scientific paper.

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Incidence of ectopic pregnancy in Texas—1979

To the Editors:

The frequency of ectopic pregnancy is usually expressed as a ratio of the total number of ectopic pregnancies to the total number of live births. A survey of Texas hospitals was conducted to determine the statewide incidence of ectopic pregnancy in 1979.

A total of 237 hospitals were contacted by questionnaire, and 153 (65%) responded. These hospitals reported a total of 1,066 ectopic pregnancies and a total of 115,744 live births during 1979. The incidence of ectopic pregnancy was 1:109 live births.

From 1944 to 1959, the incidence of ectopic pregnancies at Parkland Memorial Hospital (Dallas, Texas) was 1:123 deliveries.[1] More recently McBride and Wait[2] reported one ectopic pregnancy per 250 live births over a 6-year period in Houston, Texas. They attributed the low incidence to their large obstetric service and a relatively small gynecologic service. Both studies concerned several years' experience at one institution.

It is sometimes suggested that the frequency of ectopic pregnancy is changing. This survey provides one additional statistic. Further annual data should document any trends.

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ITEMS

Fourth Annual Mid-Winter Symposium in Obstetrics and Gynecology

The Fourth Annual Mid-Winter Symposium in Obstetrics and Gynecology will be held February 25-27, 1981, at the Scottsdale Hilton Hotel, Scottsdale, Arizona. It is cosponsored by the Phoenix Obstetrics and Gynecology Society and the Department of Obstetrics and Gynecology, Maricopa County General Hospital.

For information contact: The Secretary, Phoenix OB/GYN Society, 333 E. Virginia, Suite 222, Phoenix, Arizona 85004.

Hospital Epidemiology

Hospital Epidemiology, a regional program for physicians, will be held at the Hotel Intercontinental, Miami, Florida, January 8-9, 1981. This 2-day program will acquaint hospital epidemiologists, infection control committee chairmen, and other interested physicians with principles and practical aspects of running an infection control program. It will be presented by the APIC and APIC Dade Co. Chapter.

For further information and registration contact: Association for Practitioners in Infection Control, Rt. 2/359 Milwaukee Ave., Half Day, Illinois 60069.

Annual Meeting of the Society of Perinatal Obstetricians

The Annual Meeting of the Society of Perinatal Obstetricians will be held January 22-25, 1981, at the Hilton Palacio Del Rio, San Antonio, Texas. Submission of abstracts: Robert Sokol, M.D., Perinatal Clinical Research Center, Cleveland Metro. General Hospital, 3395 Scranton Road., Cleveland, Ohio 44109. Registration information: Donald M. Sherline, M.D., President, Society of Perinatal Obstetricians, 2650 Crabtree Lane, Northbrook, Illinois 60062.

Second International S. B. Gusberg Symposium on Reproductive Immunology

The Second International S. B. Gusberg Symposium on Reproductive Immunology will take place at The Mount Sinai Medical Center in New York, New York, on June 18 and 19, 1981, sponsored by the Department of Obstetrics and Gynecology and the Page and William Black Post-Graduate School of Medicine and the American Society for the Immunology of Reproduction.

Reports on current clinical and basic investigations in any of the four subdivisions of reproductive immunology (developmental immunology, pregnancy immunology, fertility immunology, and reproductive tumor immunology) are invited for submission in abstract form on two double-spaced pages no later than February 28, 1981. Four copies of the abstracts should be submitted to: Norbert Gleicher, M.D., Program Director, Second International S. B. Gusberg Symposium, Department of Obstetrics and Gynecology, The Mount Sinai Medical Center, One Gustave L. Levy Place, New York, New York 10029.

Specialty Review in Obstetrics and Gynecology

A Specialty Review in Obstetrics and Gynecology will be held March 30–April 4, 1981 (5½ days). It is sponsored by The Cook County Graduate School of Medicine. Course Coordinator is M. LeRoy Sprang, M.D. A.M.A. Category I Credit: 44 hours.

For further information and registration contact: 707 South Wood St., Chicago, Illinois 60612.

Second International Congress of Andrology

The Second International Congress of Andrology will be held in Tel Aviv, Israel, June 28-30, 1981.

For information contact: Medical Congress Coordinators, 1212 Avenue of the Americas, New York, New York 10036.

Fifth World Congress of Sexology

The Fifth World Congress of Sexology will be held in Jerusalem, Israel, June 21-26, 1981.

For scientific information contact: Zwi Hoch, M.D., Center for Sexual Counseling, Therapy and Education, Department of Obstetrics and Gynecology—"A," Rambam Medical Center, Haifa, Israel. For travel and scientific information and for registration contact: Medical Congress Coordinators, 1212 Avenue of the Americas, New York, New York 10036.

Sixth International Congress of Human Genetics

The Sixth International Congress of Human Genetics will be held in Jerusalem, Israel, September 13-18, 1981.

For information contact: Medical Congress Coordinators, 1212 Avenue of the Americas, New York, New York 10036.

Oral examination of American Registry of Diagnostic Medical Sonographers

The American Registry of Diagnostic Medical Sonographers will administer its oral examination in obstetrics and gynecology on April 4 and 5, 1981. Only those who passed the written examination are qualified for the oral examination.

Detailed information can be obtained from: American Registry of Diagnostic Medical Sonographers, 2810 Burnet Ave., Suite N2, Cincinnati, Ohio 45219.

Molecular Interrelations of Nutrition and Cancer

"Molecular Interrelations of Nutrition and Cancer," sponsored by The University of Texas M. D. Anderson Hospital and Tumor Institute, is the topic of the Thirty-fourth Annual Symposium on Fundamental Cancer Research to be held in Houston, Texas, at the Shamrock Hilton Hotel, March 4-6, 1981.

For additional information contact: Stephen C. Stuyck, Director, Public Information and Education, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Sixteenth Annual San Francisco Cancer Symposium

The Sixteenth Annual San Francisco Cancer Symposium, "Childhood Cancer: Triumph Over Tragedy," will be presented March 13-14, 1981, at the Hyatt Regency, San Francisco, California. It is sponsored by the West Coast Cancer Foundation in association with The California Division of the American Cancer Society.

For further information contact: West Coast Cancer Foundation, 50 Francisco St., Suite 200, San Francisco, California 94133.

Medical meetings—1981

1. Pan-American Conference on Fertility and Sterility, February 12-20, 1981, Mullet Bay, St. Maarten, N.A.
2. American College of Osteopathic Obstetricians and Gynecologists, February 14-21, 1981, Puerto Vallarta, Mexico
3. The First International Convention on Medico-Legal Aspects of Disability, February 15-19, 1981, Tel Aviv, Israel
4. Third World Congress of Human Reproduction, March 22-26, 1981, Berlin, West Germany
5. The Fifth World Congress of Sexology, June 21-26, 1981, Jerusalem, Israel
6. Second International Congress of Andrology, June 28-30, 1981, Tel Aviv, Israel
7. Second International Dermatopathology Symposium, July 12-15, 1981, London, England
8. The Sixth International Congress of Human Genetics, September 13-18, 1981, Jerusalem, Israel

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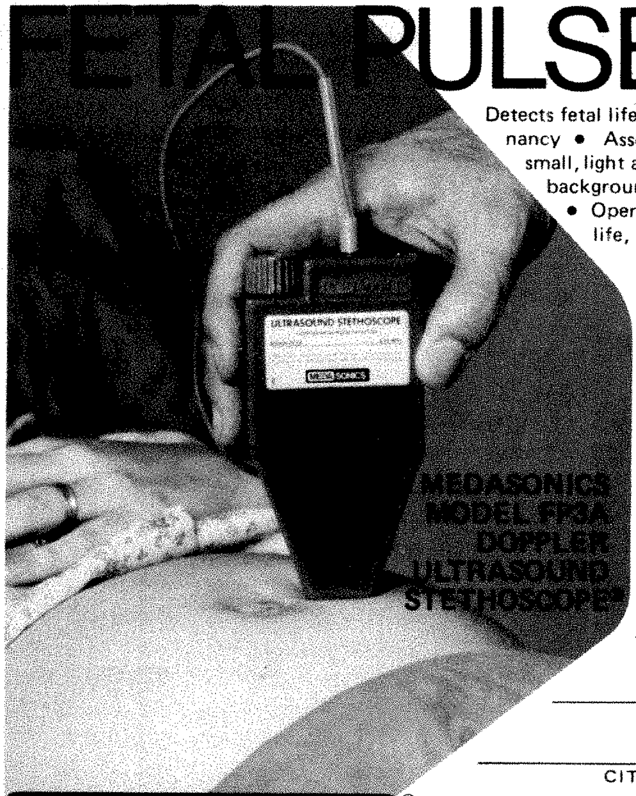

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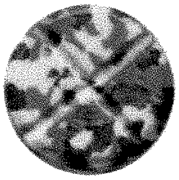

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Vol. 138, No. 7, Part 2, December 1, 1980. The American Journal of Obstetrics and Gynecology is published semimonthly by The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63141. POSTMASTER: Send address changes to above address.

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American Journal of Obstetrics and Gynecology

volume 138 *number* 7 *part* 2 DECEMBER 1, 1980

EDITORIAL

Pelvic inflammatory disease, 1980

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MOST PHYSICIANS use the term "pelvic inflammatory disease" (PID) to describe a wide variety of acute and chronic conditions resulting from ascending, traumatic, and surgical infections. Because practitioners and investigators diagnose disease using variable clinical criteria, studies are sometimes contradictory and confusing. This situation is improving. PID is now used more often to refer to diseases caused by acute, ascending genital tract infection. Researchers[1] in Sweden have developed objective case criteria using laparoscopy. Newer studies applying these criteria to identify cases are resolving some past discrepancies.

PID traditionally has been associated with gonococcal infection. Researchers investigating the microbiologic agents associated with PID now use improved techniques; in addition, they increasingly obtain specimens directly from the fallopian tubes and the pelvic cavity. These modern approaches emphasize the complex microbiology of PID, which now includes *Chlamydia*, anaerobes, and perhaps other agents in addition to the gonococcus.

Women with acute PID are seen commonly in busy hospital emergency rooms and gynecologic practices, but physicians often dismiss their illness as a nuisance rather than a serious problem. Yet Rendtorff and associates[2] demonstrate the staggering economic consequences of this preventable condition. In addition, new information confirms the importance of PID to the health and welfare of women. Other efforts to improve the health and economic status of women also contribute to recognition of the importance of PID and the need to improve control measures.

The world's leading researchers in PID and related topics met on April 1 through 3, 1980, to review many facets of this disease from their various perspectives. Over 500 distinguished scientists, clinicians, epidemiologists, microbiologists, and public health administrators from 52 countries participated in the first International Symposium on Pelvic Inflammatory Disease at the Center for Disease Control, Atlanta, Georgia. In this supplement to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, we present the papers of the Symposium to share some of the information exchanged by participants.

Consequences

In recent years, the focus in sexually transmitted diseases (STDs) has shifted from their incidence to their consequences. In this issue, Curran makes an estimate of the staggering economic costs of PID in the United States. In addition to these consequences, we should not understate the social effects of PID. PID causes

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recurrent pelvic infection, chronic pelvic pain, and infertility; family integrity is jeopardized; and individuals are unable to fill social roles. These social consequences are most serious because PID frequently occurs among women less than 25 years of age.

Risk factors

Meeting participants agreed that the relative risk of developing PID increases among women who use intrauterine devices (IUDs). Three separate reports document a decreasing risk of PID for oral contraceptive users even after correction of various other risk factors. Senanayake and Kramer urge health planners to use this information to establish programs that assist patients in selecting contraception with the least risk and the maximum benefit.

The Women's Health Study examines ectopic pregnancy and contraceptive use.[3] Overall, women who have used an IUD are at the same risk for ectopic pregnancy as those who have never used an IUD. Furthermore, current use of any form of contraceptive, including the IUD, decreases the risk for ectopic pregnancy. Among current contraceptive users, IUD users are at three times the risk for ectopic pregnancy compared with users of oral contraceptives and are at about the same risk as users of traditional contraceptives. Ory and associates[3] suggest that the IUD is not responsible for any part of the recent tripling of ectopic pregnancies in the United States.

Sinnathuray and associates, Kochar, and Begum, from three centers in Asia, report that illegally induced abortions are among the most common causes of hospitalization for pelvic infections. In striking contrast, Rushwan from the Sudan suggests that neither abortions nor STDs are common causes of PID; in that country, PID is associated most frequently with female circumcision. No specific report compared delivery practices and postpartum sepsis or PID. However, many participants feel that home delivery under less than optimal conditions is an important contributor to PID in many countries and that this subject warrants further attention.

Epidemiology

Weström reviews the information documenting the increasing incidence of PID, the increased risk for younger women, and the increased risk of PID for IUD users. In addition, he forecasts the risk of ectopic pregnancies and post-PID infertility. Weström obtained his data from developed countries; papers by Muir and Belsey and Arya and colleagues emphasize that infertility problems associated with PID may be even more severe in developing countries. Although sexually transmitted agents are important causes of PID in developing countries, other infectious agents, e.g., tuberculosis, schistosomiasis, and filariasis, are important causes also. Holmes and colleagues hypothesize that the widely divergent views of PID epidemiology are caused at least partially by true differences in etiologic and epidemiologic factors in different populations.

Analysis of hospitalizations for PID in the United States by Jones and associates emphasizes the importance of this problem and supports the hypothesis that PID is sexually transmitted. O'Hare and co-authors emphasize that hospital emergency rooms are a primary center for diagnosis, treatment, and management of PID and must be an integral part of control efforts in the United States. Adler analyzes the reporting systems for PID cases in England and Wales and indicates improvements in data collection which will increase our understanding of disease epidemiology.

Microbiology

Different interpretations of the microbiologic causes of PID generate heated controversy. In the past few years, better microbiologic techniques, increased use of specimens collected directly from pelvic organs, and new developments in serologic tests have increased understanding of this area. Mårdh extensively reviews epidemiologic, clinical, microbiologic, serologic, and animal model studies, explains some of the controversy, and firmly establishes the important roles of *Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma hominis,* and anaerobic organisms in the pathology of PID. Groups led by Sweet, Ripa, Paavonen, Ratnam, and Perine document the importance of different microbiologic agents in patients with PID studied in different centers.

Recent observations on the basic pathophysiology of PID and details of organism-tissue interactions are presented by groups of authors headed by Gregg, Sweet, Draper, Chow, and Møller. These investigators report several in vitro and in vivo models used to study infecting agents, routes of infection, and tissue responses. Finally, Buchanan and colleagues state that serotype-specific immunity to recurrent gonococcal PID may result from a prior gonococcal PID infection; the authors hypothesize that a vaccine could be developed to protect against this complication.

Clinical spectrum

The clinical symptoms and signs of PID are highly variable, and the difficulties in making an accurate differential diagnosis are well known. Jacobson, in his review of diagnostic problems, evaluates the accuracy of available diagnostic tests and suggests that laparoscopy is essential for accurate diagnosis of PID. Ledger, however, considers the difficulties posed by widespread use of laparoscopy and advocates selective use to improve diagnostic certainty, evaluate the impact of therapy on prognosis, and improve current microbiologic knowledge. Cooperman and Ruiz describe PID cases in a major metropolitan hospital in the United States; nearly half their patients had a pelvic mass or peritonitis. Svensson and associates describe clinical observations of genital chlamydia infections. Clinical syndromes newly associated with *C. trachomatis* infection include "silent PID" among infertile women reported by Henry-Suchet and co-workers; intrapartum and late postpartum fevers described by Wager and colleagues; and the Fitz-Hugh–Curtis perihepatitis syndrome as detailed by Wang and co-authors. Finally, Hagenfeldt and Szanto suggest that sacroiliitis frequently follows acute severe PID.

Treatment

Diagnostic difficulties and the variety of microorganisms which cause PID pose serious problems for investigators studying antimicrobial treatment. In her review of treatment of pelvic infections, Rees stresses the need for accurate microbiologic diagnosis as the basis for proper selection of antimicrobial agents. Furthermore, she reports that some cases of nongonococcal PID are prevented by using tetracycline to treat uncomplicated gonococcal infections in women. The need for carefully designed therapeutic studies remains. Bell and James present a theoretical model that assesses the potential of several hundred antibiotic combinations. This approach provides a rational basis for selecting regimens for study. Ginsburg and his co-authors review the course of patients treated for tubo-ovarian abscesses and find that current therapies are unsatisfactory. Duncan and her group share their experiences in the treatment of puerperal sepsis and PID in Ethiopia. In developing countries therapy is more difficult because abscesses and sepsis are more common.

Prevention and control

Meheus provides a framework for considering the problems of PID prevention and control. His discussion of primary, secondary, and tertiary prevention of PID and its sequelae provides a logical beginning for the design and implementation of STD control policies. Rajan, de la Torre, Werthein and colleagues, Nsanze, and Osoba from the Far East, South America, Cuba, and Africa, respectively, describe the many problems that must be surmounted to initiate PID prevention and control. Brown and Wiesner describe the evolution in the United States of the gonorrhea control program into a gonococcal PID prevention program and they suggest that these efforts will lead eventually to control efforts for chlamydial PID. Meheus warns that current gonorrhea prevention and control strategies are yet to be demonstrated as effective for control of *C. trachomatis* infections.

Both Potterat and associates and Curran and coworkers reemphasize that male sexual consorts of women with gonococcal PID frequently are asymptomatic. These authors suggest that such men may be important in gonorrhea transmission and may reinfect their sexual partners, causing recurrent PID. Cooperative efforts between public health departments and hospital emergency rooms are feasible and should reduce the consequences of this illness.

Finally, Spence and his co-authors describe the clinical services necessary to ensure adequate diagnosis and follow-up in PID patients and to train professional personnel.

We now recognize the importance of this disease for women and society. New investigators have begun important studies; governments have committed additional resources for prevention and control. Yet individual clinicians in STD clinics, family practices, and specialties such as obstetrics-gynecology and emergency medicine must play the major role in preventing PID and limiting its consequences. To prevent PID they must identify, examine, and treat both the female partners of men with STD and women at high risk of having an STD. To prevent the consequences of PID, they must diagnose the condition promptly and accurately, begin effective treatment immediately, counsel all patients regarding the nature and importance of their illness, and ensure the examination and treatment of male sexual partners.

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ECONOMIC CONSEQUENCES

Economic consequences of pelvic inflammatory disease in the United States

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Pelvic inflammatory disease is the most common serious complication of sexually transmitted infections caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Initial episodes of sexually transmitted acute PID occur most frequently in the 20 to 24 year age group, while sequelae such as infertility, ectopic pregnancy, and chronic PID are most common among 25- to 34-year-old women. The apparent chronicity of the disease process suggests that the epidemic of sexually transmitted disease, which began in the mid-1960s, could be followed by an epidemic of PID and its sequelae. More than 850,000 episodes of PID occur annually, requiring more than 212,000 hospital admissions, 115,000 surgical procedures, and 2,500,000 physician visits. Ectopic pregnancies in the United States tripled from 1967 to 1977. Direct and indirect costs for PID and PID-associated ectopic pregnancy exceeded $1.25 billion in 1979. If PID and ectopic pregnancy rates continue unabated, by the year 2000 there will have been more than one episode of PID and three related physician visits for every two women who reached reproductive age in 1970. Fifteen percent will require hospitalization, more than 3% will experience an ectopic pregnancy, and more than 10% will involuntarily become sterile because of PID. (AM. J. OBSTET. GYNECOL. 138:848, 1980.)

PELVIC INFLAMMATORY DISEASE in women is the most common serious complication of infections caused by the sexually transmitted pathogens *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Although these organisms are not isolated from all women with PID, the syndrome is rare in women who are not sexually active.[1, 2] The gynecologic history in many women with PID suggests a chronic disease process. The initial episodes of acute PID occur most commonly in single young women in the age group at highest risk of contracting gonorrhea and other sexually transmitted diseases (STDs).[3-7] With few exceptions, symptoms in these patients disappear quickly after antibiotic therapy. Recurrent or relapsing PID is common, but sexually transmitted organisms are isolated less frequently during these recurrent episodes.[8, 9] The majority of hospital admissions and surgical procedures for PID occur among married women in the 25 to 34 year age group.[7, 10] PID caused by sexually transmitted pathogens results in infertility in more than 20% of cases[3] and the risk of ectopic pregnancy increases six- to tenfold after PID.[3, 11] Hospitalizations for infertility and ectopic pregnancy also are most common among married women aged 25 to 34 years.[12, 13]

From 1965 to 1975, the number and rate of reported cases of gonorrhea tripled; since 1975, reported cases have leveled off at one million per year. Surveys indicate that nongonococcal urethritis in men and, presumably, genital chlamydial infections in women also were increasing rapidly during this period.[14]

If we assume that the STD epidemic, which began in the mid-1960s, was accompanied by an increased incidence of PID, then the proportion of women in the "post-PID" state will be increasing in the population. With time and later attempts at childbearing, the chronic sequelae of PID, such as recurrent infection, infertility, and ectopic pregnancy, will be recognized. In this article, I will discuss the latest available data on

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the occurrence of these disorders and estimate the costs of PID in the United States for 1979.

Involuntary infertility

No accurate information on the extent of STD or PID-associated infertility exists. The National Surveys of Family Growth examined fertility impairment and contraceptive status among married and previously married women aged 15 to 44 years in 1973 and again in 1976.[15-17] Among these married or previously married women, there was a 45% annual increase (i.e., an increase of 122,000 couples per year) in the estimate of nonsurgical sterility between the two surveys. Although differences in survey methodology or better diagnosis of infertility may explain the change, the occurrence is compatible with an epidemic of STD-associated PID. In the 1976 survey, 34% of "noncontracepting" married or previously married women classified themselves as "noncontraceptively" sterile,[15, 16] about 15% of these for nonsurgical reasons.

PID occurrence

The occurrence of PID has recently been estimated. From the Hospital Discharge Survey conducted between 1970 and 1975, there was an estimated annual average of 212,611 hospital-discharged patients with diagnoses of PID or salpingitis.[7, 10] PID or salpingitis was the primary diagnosis in approximately 65% of cases. An additional 15% of patients had primary diagnoses probably related to PID, such as "infertility" or "peritonitis"; in fewer than 4% of the cases PID was clearly an incidental diagnosis. An annual average of 115,350 surgical procedures were performed in patients with PID and hospital days totaled 1,594,000 annually. An average of 897 of these hospitalized women died each year.

Data on outpatient visits for PID from 1961 to 1977 have been reported by St. John and associates.[18] From 1961 to 1974, estimates were made from data supplied by the National Disease and Therapeutic Index (NDTI) probability sample of office-based physicians, and from 1973 to 1977, data from the National Ambulatory Medical Care Survey (NAMCS) were used for estimation.[18] The earlier NDTI data suggest a marked increase in PID outpatient visits from 1970 to 1973. Though the authors caution that NDTI and NAMCS data are not directly comparable, they suggest that visits to office-based physicians for PID may have declined between 1973 and 1974, in part as a result of intense gonorrhea screening activities initiated at that time. For purposes of cost estimates I have used the more recent NAMCS data.

Table I. Incidence of ectopic pregnancy in the United States,[11, 20] 1967 to 1977

Yr	*Ectopic pregnancies*	*% Change from previous year*	*Rate**
1967	13,200	—	NA
1968	18,700	+41	NA
1969	NA†	—	NA
1970	17,900	−02	NA
1971	19,300	+08	4.77
1972	24,500	+27	6.37
1973	25,800	+05	6.87
1974	26,800	+04	6.83
1975	30.800	+15	7.70
1976	34,600	+12	8.32
1977	41,100	+19	9.35

*Number of ectopic pregnancies per 1,000 live births, plus legal abortions.

†Not available.

The NAMCS data indicate that women with PID annually averaged nearly 1.9 million outpatient visits to office-based private physicians between 1973 and 1976. Visits to office-based private physicians account for three quarters of visits to physicians annually; poor, young black women are more likely than others to seek care from outpatient clinics and hospital emergency rooms rather than from private physicians.[19] Sex- and race-specific extrapolations for attendance at these facilities would indicate that an additional 680,000 physician visits for PID occur annually in clinics and emergency rooms.[19] Thus, there was an annual average of 2,572,000 outpatient physician visits for PID between 1973 and 1976. If we assume that women with PID averaged three visits per episode, 857,000 episodes of PID occurred annually between 1973 and 1976.

Ectopic pregnancy

Ectopic pregnancies tripled in number from 1967 to 1977[11, 20] (Table I). The annual increase is reminiscent of the annual rise in reported gonorrhea during the late 1960s and early 1970s. In 1977, ruptured ectopic pregnancy accounted for 12% of maternal deaths and was the leading cause of maternal death among nonwhite women in the United States.[21] Studies implicate existing PID as the cause of approximately half of ectopic pregnancies[22-24]; therefore, in estimating costs I attributed 50% of the morbidity and deaths from ectopic pregnancy to PID. To the extent the recent increased incidence is secondary to PID, this represents an underestimate.

Economic costs of PID

To estimate the economic costs of PID, the incidences of PID and ectopic pregnancy in 1979 are assumed to be the same as in the most recent aforementioned data. Klarman[25, 26] divides costs into direct, indirect, and intangible. Direct costs are those associated with spending for health services. Indirect costs refer

Table II. PID and associated ectopic pregnancy in the United States—direct costs, 1979

PID	
Hospital admissions	$398,650,000
Surgical procedures	86,512,500
Outpatient expenses	128,650,000
Other direct costs	30,690,600
Subtotal	$644,503,100
Ectopic pregnancy (50% of total)	
Hospital admissions	$ 35,448,750
Surgical procedures	15,412,500
Outpatient expenses	1,027,500
Other direct costs	2,594,400
Subtotal	$54,483,150
Infertility caused by PID	(Not estimated)
Total direct costs	$698,986,250

Table III. PID and associated ectopic pregnancy in the United States—indirect costs, 1979

Output losses	
Inpatient PID	$216,055,300
Outpatient PID	218,459,600
Ectopic pregnancy	20,882,900
Subtotal	$455,397,800
Mortality losses	
Inpatient PID	$ 99,394,400
Ectopic pregnancy	2,544,200
Subtotal	$101,938,600
Total indirect costs	$557,336,400

to output lost due to disease and to losses from premature death. Since incomes for women are generally lower than those for men and since women are less likely to be employed, diseases affecting women, such as PID, tend to be relatively undervalued. In the future, as the number of women working increases so will the indirect costs for diseases primarily affecting women increase. To partially offset this present undervaluation, many economists favor placing a value on housewives' services.[26-28] We computed output lost by women of various age groups by adapting the approach suggested by Cooper and Rice.[28] We computed daily earnings for women, taking into account the percent of the work force employed and adapting a market value for housewives' services. For computation purposes, work losses caused by PID and ectopic pregnancy were distributed in the 20 to 34 year age groups. Thirty days of lost output were assumed for hospitalized women and 10 days per episode for outpatients.

Many precedents exist in health economics regarding the valuation of life lost. We estimated economic losses from premature death from the 1972 sex-adjusted table of Cooper and Rice.[28] The underestimate from use of a 1972 table is partially offset by the low 4% discount rate. We calculated mortality losses for ectopic pregnancy from the 25 to 29 year age group, and for PID from the 25 to 34 year age group.

We placed no dollar value on the intangible costs of PID such as suffering, deprivation of parenthood, marital discord, and school absenteeism; therefore, the total is underestimated to that extent. Since extensive health resources are allocated for reconstructive tubal surgery and in vitro fertilization, the direct and indirect costs of PID-induced sterility are considerable. Unfortunately, the data on the incidence of PID-induced sterility and the extent of remedial operations necessary to estimate direct and indirect costs are unavailable.

Estimates used for computing 1979 direct costs for hospitalization, gynecologic surgery, and outpatient visits were rounded off from the most recent data available from the Health Care Financing Administration.[29, 30] Daily hospitalization, including testing and medication, was tabulated at $250, while gynecologic surgery and anesthesiology charges were computed at $750. The cost of an outpatient visit for PID, including testing and medications, was tabulated at $50. Since incidental costs increased direct costs of PID by more than 5% in an earlier study in Memphis,[8] this amount was added herein as "other direct costs."

In 1979, direct costs (Table II) of PID and associated ectopic pregnancy reached nearly $700 million and indirect costs more than $555 million (Table III). Total costs for PID in 1979 are estimated at $1,256,322,600.

Ruptured ectopic pregnancy invariably results in fetal death. Though placing an economic value on a fetal life is somewhat controversial, considerable grief and loss of human potential do result from ectopic abortion. In the absence of suitable precedents, I chose to compute fetal mortality losses as would be done for infants less than 1 year of age. The losses from these 20,500 fetal deaths from PID-associated ectopic pregnancy result in an additional $1,461,000,000 in indirect costs of PID. If this fetal mortality estimate is included, total costs of PID for 1979 exceeded $2.7 billion.

Discussion

Annually, nearly one million women in the United States suffer from PID and its sequelae. They account for more than 2.5 million physician visits, 250,000 hospital admissions, and nearly 150,000 surgical procedures. STD-associated PID often begins in young, single women; manifestations of recurrent disease, sterility, ectopic pregnancy, and major surgery occur 5 to 10 years later.

Between 1965 and 1975, gonorrhea infections increased dramatically in the United States. Although trend data on PID occurrence are not adequate to allow firm conclusions regarding similar increases in PID, the NDTI data suggest an increase in office-based physician visits beginning about 1970. Furthermore, ectopic pregnancy, a known sequel to PID, steadily increased in incidence throughout the 1970s. What lies in store for the current generation of young women if the high rates of STD, PID, and ectopic pregnancy continue? By the year 2000, there will have been one episode of PID and three physician visits for PID for every two women who reached reproductive age in 1970. Fifteen percent of this group will have been hospitalized for PID, over half of these requiring major gynecologic surgery. If the current 20% risk of infertility after a PID episode continues, over 10% of these women will have been sterilized by PID alone, representing a doubling of the current estimate of prevalence of nonsurgical sterility. Finally, 3% of this reproductive generation of women will experience an ectopic pregnancy.

Taxpayers will continue to share the burden as the economic costs of PID escalate. Recognition by our society of the medical, social, and economic consequences of PID could improve this outlook. Intensification of research efforts in the infectious epidemiology, pathogenesis, and management of PID is critical to the ultimate solution. Currently, however, more careful diagnosis and management of patients with PID and prevention and early treatment of gonorrhea and chlamydia infections could reduce the occurrence of PID and improve the prognosis for patients with this disorder.

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Contraception and the etiology of pelvic inflammatory disease: New perspectives

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The dramatic increase in use of contraception worldwide makes it imperative to understand the effects of contraceptives on the health of women using them. In this article, we review the literature on the relationships of modern nonsurgical contraception with pelvic inflammatory disease. Subsequently, we identify areas where further research is needed to better define the risks and benefits of these contraceptive methods in various settings. From our review, two new conclusions emerge. First, our reanalysis of published data on the risk of PID associated with intrauterine device (IUD) use compared with no contraceptive use shows, with one exception, less risk than the previous comparisons to all non-IUD use. Second, and probably most importantly, the studies, when taken together, strongly imply that oral contraceptives have a protective effect against PID. Only one study of 11 is equivocal on this point. (AM. J. OBSTET. GYNECOL. 138:852, 1980.)

THE DRAMATIC increase in use of contraception worldwide makes it imperative to understand the effects of contraceptives on the health of women using them. In this article, we review the literature on relationships of modern nonsurgical contraception with pelvic inflammatory disease. Subsequently, we identify areas where further research is needed to better define the risks and benefits of these contraceptive methods in various settings.

Epidemiologic studies on the intrauterine device–PID relationship

To date, there have been at least 16 published reports of controlled epidemiologic studies designed to illuminate the relationship between intrauterine device (IUD) use and acute PID. These studies were conducted by 14 different investigators in five different countries using several different study designs. In all of these studies, use of the IUD was found to carry an increased risk of PID when compared with nonuse of the IUD (Table I). In a previous review, Ory[1] found the best estimate of increased risk of PID for IUD users compared with nonusers to be three- to fivefold. Inclusion of studies published subsequent to that report and analyzed in a similar fashion has not altered that estimate. However, some of these studies have been performed and reported in such a way that we have been able to reanalyze the data to evaluate the relative risk of PID with IUD use compared with non-IUD, non–oral contraceptive use. Unfortunately, many of the studies do not separate nonusers of contraceptives from users of barrier methods, and in these cases, we have been

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obliged to include the barrier users in our contraceptive nonuser group. However, by segregating the users of hormonal contraceptives in our reanalyses, we have eliminated their confounding effect.

Studies headed by Wright,[2] Lippes,[3] Weström,[4] Vessey,[5] Thaler,[6] Flesh,[7] and Kaufman[8] all indicate a relative risk of acute PID for IUD users compared with all others that is in or near the range of Ory's estimate. One study by Phaosavasdi and associates[9] found a much higher relative risk; however, the study design actually examined the risk of both acute and past inflammation, and the addition may be sufficient to explain the difference. The published articles cited above do not provide data that can be analyzed to compare IUD users with nonusers of contraceptives. The studies which do provide these data are described in the text and in Table I.

Noonan and Adams[10] studied the indigent population of Atlanta, Georgia, using a relatively nonspecific case definition for PID. From a systematic sample of family planning clinic patients, they chose 1,706 women who were then screened for cervical gonorrhea and questioned for a history of PID symptoms. Women with IUDs had symptoms of PID 2.5 times as frequently as women not using any contraception and 1.8 times as frequently as women using all other methods of contraception combined.

Targum and Wright[11] studied first-episode acute PID in 50 low-income, black and Hispanic patients, mostly inpatients at a major New York medical center.[11] Two sets of outpatient controls were chosen by matching for age, marital status, and interval since last pregnancy. In this study there was a relative risk of PID of 7.3 for IUD users compared with nonusers of contraceptives, and 9.3 compared with non-IUD users. The risk for IUD users in this study is the highest reported in any of the studies and probably is due in part to hospitalization bias, i.e., most patients with PID (80%) were hospitalized but none of the controls was hospitalized. If IUD users with PID are hospitalized more often than non-IUD users with PID, or if IUD use is recorded more reliably for inpatients than for outpatients, the relative risk of IUD use would be elevated. Additionally, in this study there was a disproportionate number of users of the Majzlin spring, which was recalled by the United States Food and Drug Administration because of high rates of complications.

Faulkner and Ory[12] reported a prospective case-control study done in Atlanta. To increase specificity in the diagnosis of PID they used an oral temperature of 38° C as an extra objective criterion. Cases were stratified into febrile and afebrile PID. Among the women with cases of febrile PID, 38% were wearing IUDs; among women with afebrile PID, 24% were wearing IUDs; and among controls, 11%. The age-standardized relative risk of afebrile PID was 2.7, while the febrile PID risk was 5.1 for IUD users compared with nonusers. In IUD users compared with nonusers of contraceptives, the relative risk was 3.7 for febrile PID. Stratification by febrile or nonfebrile illness was the method of handling possible bias occurring when physicians were more likely to diagnose PID in women with vague abdominal pain and wearing IUDs. The results showed the association between PID and IUDs to be stronger with the addition of the temperature criterion, indicating that physician ascertainment bias did not occur. Controlling for frequency of sexual intercourse or number of sexual partners did not alter the relative risk.

Eschenbach and colleagues[13] analyzed 173 matched pairs of PID patients and controls in Seattle. Matching in this study was for age, race, marital status, presence or absence of gonococcal infection, parity (nulligravid or not), number of sexual partners, and number of previous abortions. By use of the ratio of discordant pairs, the authors calculated the relative risk for IUD users compared with nonusers of contraceptives to be 4.0. For IUD users compared to non-IUD users, the relative risk was 4.4. This study also addressed the risk of IUD use in nulligravid women and found an increased risk with an IUD in place.

The Combined Bangkok Hospital Group and Wright[14] examined 1,583 consecutive women undergoing laparoscopic sterilization procedures. Of that group, 9.5% had evidence of previous PID. Women who had never practiced contraception had an 8.9% prevalence of past PID, while women who had used only the IUD had a 12.3% prevalence of past PID. Thus, in this series, the relative risk of PID for women using an IUD compared to women not using contraception was 1.4. As pointed out by the authors, data from this study may be biased because of self-selection of the cohort that was examined. Almost 90% of the women had used a contraceptive method at some time, while 71% were current users. These proportions differed from those reported for married women under 45 years of age in Bangkok in 1973.[15] Moreover, women undergoing sterilization procedures are different from the general population of women, perhaps being better educated or having better access to health care. Additionally, comparisons by method were made with continuous users of the method compared with nonusers of any method.

Since the incidence of IUD-related PID decreases

Table I. Summary of epidemiologic studies on the IUD-PID relationship

Type of study	*No. of subjects*	*RR-IUD vs. all other contraception* (95% CI†)*	*RR-IUD vs. no contraception* (95% CI†)*	*Comments*
Historical prospective (Wright and Laemmle[2])	108	4.2 (not applicable)		Case ascertainment in defined population; method-specific denominator estimates
Prospective cohort (Noonan and Adams[10])	1,706	1.8 (1.5-2.1)	2.5 (1.8-3.5)	Systematic sample of family planning clinic patients; nonspecific case definition for PID
Prospective case-control (Targum and Wright[11])	150	9.3 (4.2-20.9)	7.3 (2.9-18.4)	Hospitalization bias: most patients hospitalized, all controls outpatients; excess use of Majzlin spring; first-episode acute PID only
Retrospective case-control (Lippes[3])	129	1.9 (0.5-7.2)		Small number of controls (38) limits strength of association
Retrospective cohort (Phaosavasdi et al.[9])	200	9.2 (3.7-22.5)		Nonspecific definition of previous or current PID diagnosed visually at sterilization; 15% prevalence rate of PID in cohort; method of selection of cohort not specified
Prospective case-control (Faulkner and Ory[12])	250	5.1‡ (2.5-10.5)	3.7 (1.7-8.2)	Strict criteria for PID increased risk of PID in IUD users
Prospective case-control (Weström et al.[4])	1,256	2.9§ (2.1-4.0)		Exclusion of many young nulliparous women in control group; elevated risk for nulliparous women at all age strata

*Relative risks (RR) calculated by the method of Mantel and Haenszel[35]; 95% confidence intervals (CI) in parentheses calculated by method of Miettinen.[36]

†Confidence interval of 95% which does not include 1 indicates statistical significance for calculated relative risk.

‡Standardized for age.

§Standardized for age and parity.

‖Comparison group includes only oral contraceptive and diaphragm users.

¶Calculated by ratio of discordant pairs.

#Relative risk based on continuous use of only one method.

**PID defined as edema and congestion or acute inflammatory infiltrate on histologic tissue examination of tubes.

over time,[16] and presumably also decreases when the IUD method is discontinued, the kind of stratification used in this study would tend to move the relative risk toward unity (in this case, to minimize it).

Maqueo and colleagues[17] performed histologic examinations of fallopian tube tissue obtained during sterilization procedures on 225 women in Mexico. This cohort was chosen to include 75 normally cycling women, 75 women 2 to 3 days post partum, and 75 asymptomatic women who had used a nonmedicated IUD for at least 1 year. The frequency of tubal changes including edema, congestion, and acute inflammatory infiltrate was 45% in IUD users and 35.6% for nonusers of contraceptives. The relative risk of having acute inflammatory changes for IUD users compared with nonusers of contraceptive is 1.5. Evaluation of the intensity of the acute inflammatory infiltrate showed 25% of the women with IUDs had marked inflammation, while only 4.5% of the nonusers of contraceptives had marked inflammation. Neither of these differences is statistically significant. However, in both physiologic parameters, the direction of the difference is the same as that in the other studies, i.e., the IUD carries a greater risk of inflammation and of more severe inflammation. Three factors in the design of this study may have contributed to the low relative risk and the lack of statistical significance in the results. First, a very small number of subjects was studied, thus limiting the power of the statistical test. Second, all IUD users had had their IUDs in place for at least 1 year. Several studies[16, 14] have shown decreasing overall PID rates with increasing duration of IUD use, so that excluding women who had used a device for less than 1 year would lower the risk relative to that shown in other studies without a similar exclusion. Third, the case definition for PID was nonspecific.

In a 1979 study done by Ryden and colleagues[18] in Sweden, a total of 87 women with laparoscopically confirmed PID and positive gonorrhea cultures were compared with 174 women with positive gonorrhea cultures but without PID. The control group was comparable in age, marital status, and sexual activity to the

Table I—cont'd

Type of study	*No. of subjects*	*RR-IUD vs. all other contraception** *(95% CI†)*	*RR-IUD vs. no contraception** *(95% CI†)*	*Comments*
Prospective cohort (Vessey et al.[5])	17,032	3.5‖ (2.1-6.1)		Nearly 97% follow-up; based on inpatient illness
Prospective case-control (Eschenbach et al.[13])	346	4.4¶ (3.0-6.9)	4.0¶ (not available)	Extensive matching; outpatient interviews; nulligravid women are at higher risk; IUD use associated more strongly with nongonococcal PID than with gonococcal PID
Prospective case-control (Thaler et al.[6])	202	2.3 (0.9-5.9)		Population from which controls drawn unspecified; first-event acute PID only
Prospective case-control (Flesh et al.[7])	345	2.2 (1.3-3.9)		Hospitalization bias: hospitalized patients, outpatient controls; cases not excluded because of surgical sterility or sexual inactivity, controls excluded resulting in conservative estimate of RR
Prospective cohort (Combined Bangkok Hospital Group[14])	1,583	2.1# (1.3-3.3)	1.4# (0.8-2.5)	Examination of self-selected cohort undergoing laparoscopic sterilization
Prospective case-control (Maqueo et al.[17])	225		1.5** (0.8-2.8)	Patients used IUD for at least 1 year; evidence of current inflammation of tubes by pathologic examination after sterilization in asymptomatic women
Prospective case-control (Ryden et al.[18])	261	2.3‡ (1.2-4.6)	1.7‡ (0.8-3.6)	Cases and controls from alphabetically arranged files of women with gonorrhea; PID confirmed by laparoscopy; cervical gonorrhea more common in women with PID than women with uncomplicated gonorrhea
Prospective case-control (Kaufman et al.[8])	303	7.9§ (3.7-16.9)		Hospitalized women only; all controls used either IUD or oral contraceptives; first-episode acute PID only
Retrospective case-control (Osser et al.[19])	1,380	2.0§ (1.6-2.6)	1.6 (1.2-2.1)	Cases hospitalized, outpatient controls; parity did not change RR; matched pair design without matched pair analysis

PID group. This is the only study for which the original publication showed a comparison between IUD users and nonusers of contraceptives; the relative risk that we calculated from the published data is 1.7. In this study, there is once again the problem of a small number of subjects. Additionally, this study differs from the others in the fact that all women had positive gonorrhea cultures. Evidence for the possible effect of gonorrhea on the relative risk for IUD users is conflicting and will be discussed later; however, the study still shows an increased risk of PID for IUD wearers. While this relative risk is not significantly different from that for nonusers of contraceptives, the lack of statistical significance does not invalidate the finding of an elevated risk.

The most recent published study was a retrospective case-control study performed by Osser and colleagues[19] in Sweden. The cases included 690 women hospitalized for PID over a 3 year period. The controls were 690 nonhospitalized, sexually active women matched for age and chosen via a mailed questionnaire. The relative risk for IUD use compared to no contraceptive use was 1.6, the lowest statistically significant increase in risk shown in any of the studies. The low risk is particularly interesting in light of the hospitalization bias in this study which, as discussed earlier, should tend to increase the relative risk. However, a counterbalance to this was the nonuse of matched pair analysis, despite matched pairs in the study design. Although this does not change the direction of the relative risk, it does bring it closer to unity.

Epidemiologic studies of the oral contraceptive-PID relationship

Eleven published studies include data on the relative risk of developing PID for oral contraceptive users,[4, 8-14, 18-20] and seven of these studies compare oral contraceptive use to nonuse of any contraceptive.[10-14, 18, 19] With one exception,[10] these studies show a relative risk of PID of 0.3 to 0.9 for oral contraceptive users compared to nonusers of contraceptives, and even lower relative risks compared to users of all other

Table II. Summary of epidemiologic studies on oral contraceptive (OC)–PID relationship

Type of study	*No. of subjects*	*RR-OC vs. all other contraception* (95% CI†)*	*RR-OC vs. no contraception* (95% CI†)*	*Comments*
Prospective cohort (Noonan and Adams[10])	1,706	0.8 (0.7-1.0)	1.6 (1.1-2.3)	See Table I
Prospective case-control (Targum and Wright[11])	150	0.6 (0.3-1.3)	0.9 (0.4-2.3)	See Table I
Retrospective cohort (Phaosavasdi et al.[9])	200	0.2 (0.1-0.5)		See Table I
Prospective case-control (Faulkner and Ory[12])	250	0.4 (0.2-0.8)	0.6 (0.2-1.3)	See Table I
Prospective case-control (Weström et al.[4])	1.256	0.2 (0.2-0.3)		See Table I
Prospective case-control (Eschenbach et al.[13])	346	0.4‡	0.3‡ (not available)	See Table I
Prospective cohort (Royal College of General Practitioners[20])	46,377	0.5§ (not available)		Observations from 1,400 general practitioners; separate data for IUD users not available; no uniform diagnostic standard for PID
Prospective cohort (Combined Bangkok Hospital Group[14])	1,583	0.5‖¶ (0.3-0.7)	0.6‖¶ (0.3-1.0)	See Table I
Prospective case-control (Ryden et al.[18])	261	0.5‖# (0.3-0.8)	0.6‖# (0.3-1.1)	See Table I
Prospective case-control Kaufman et al.[8])	303	0.2#** (0.1-0.3)		See Table I
Retrospective case-control (Osser et al.[19])	1,380	0.3 (0.3-0.4)	0.4 (0.3-0.5)	See Table I

*Relative risks (RR) calculated by the method of Mantel and Haenszel[35]; 95% confidence intervals (CI) in parentheses calculated by method of Miettinen.[36]

†Confidence interval of 95% which does not include 1 indicates statistical significance for calculated relative risk.

‡Calculated by ratio of discordant pairs.

§Ratio of standardized rates, $p < 0.05$.

‖Includes all hormonal contraception rather than only oral contraceptives.

¶Relative risk based on constant use of only one method.

#Standardized for age.

**Compared to IUD users only.

methods of contraception. Most of these studies, including all those that compare oral contraceptive use to no contraceptive use, have been discussed previously herein, and their strengths and weaknesses have been pointed out. The following section discusses studies that are relevant to oral contraceptives and PID and that have not yet been reviewed, and also points out some further aspects of the study which differ in conclusion from the others.

The study by Phaosavasdi and colleagues[9] utilizes a retrospective cohort design on data obtained by chart review on a series of women who had undergone sterilization procedures. In this study, oral contraceptives were grouped with other hormonal methods, and the relative risk of PID is 0.2 for users of the hormonal methods compared to users of all other contraception. Compared to other studies, this relative risk is particularly low. The nonspecific case definition probably includes cases of sterile inflammation associated with IUD use.[21, 22] Thus, in this study there is an increased relative risk with IUD use and, by comparison, a consequent decrease in relative risk with hormonal contraception. Additionally, the method of selecting the cohort is not clear, and there may be some selection bias.

Weström and colleagues[4] reported a very large case-control study of PID in Sweden, using essentially all cases of PID occurring in a circumscribed geographic area between 1971 and 1975, for a total of 515 cases of laparoscopically diagnosed PID. From a systematic sampling of healthy women in the same area, 1,545 women were selected as controls, 48% of whom eventually participated. A large proportion of those excluded were young nulliparous women. The relative risk for oral contraceptive use compared to all other contraceptive use was 0.2. This, again, is a very low relative risk. Although no data are available as yet on the possible confounding effects of age and parity on the risk of PID for oral contraceptive users, the exclusion of a large number of young nulliparous women from the control group probably increased, by comparison, the relative risk for IUD users and consequently decreased the risk for oral contraceptive users.

The only study to address solely oral contraceptive use was performed by The Royal College of General Practitioners,[20] U. K., which published an extensive volume on oral contraceptive use. Data were collected by 1,400 general practitioners, and based on 34,875 woman-years of pill use, standardized for age, parity, social class, and cigarette smoking, the study found a PID rate of 0.42 per 1,000 woman-years. The ratio of standardized rates of PID for oral contraceptive users compared with nonusers is 0.5 and is statistically significant. In this study, no uniform diagnostic standards for PID were used; therefore, cases may or may not have been strictly defined by any particular practitioner. Any reporting bias probably would be in favor of an increase in cases among oral contraceptive users, since they see their physicians on a more regular basis than nonusers[20] and might be inclined to complain of symptoms at that time.

Kaufman and colleagues[8] did a case-control study on hospitalized women. Cases were limited to first-episode acute PID only, and all women were sexually active. The age-adjusted relative risk of PID was 0.2 for oral contraceptive users compared to IUD users. Unfortunately, the study population was limited to only those sexually active women using either IUDs or oral contraceptives. No other comparison group was included. This produces a spuriously low relative risk for oral contraceptive users, as well as a spuriously elevated risk for IUD users (Table I and II).

The Noonan and Adams[10] study discussed previously is the only study to show an increased risk of PID for oral contraceptive users. The probable explanation for the inconsistency lies in the case definition for PID, which includes nonspecific, nonmenstrual pelvic or abdominal pain. In this study higher rates of these symptoms occurred in users of oral or barrier contraceptives than in nonusers. Clearly, this is an unusual clinical finding. But consequent to it, the rate of PID defined by these symptoms probably was unusually high for oral contraceptive users, resulting in a spuriously elevated relative risk of PID.

Discussion

Taken together, the reported studies on the IUD and PID deal with most of the biases that could explain the apparent increases in risk with the IUD. Results of the studies are consistent in direction despite varying study designs, researchers, and locations, and they generally point to an increased risk of 150% to 400% for IUD users when compared with all nonusers of contraceptives. Conversely, analyses on the many studies in which the influence of oral or, in some cases, any hormonal contraceptive use can be filtered from the results show a protective effect for women using these contraceptives. The relative risk for these women compared to women using no contraception ranges from 0.3 to 0.9, with a median of 0.6.

Our review shows the relative risk of PID with IUD use to be lower than past estimates. When IUD use is compared with no contraception, the relative risk is 150% to 400% higher, while comparison with all non-IUD contraception shows a three- to fivefold increase. Although the difference in relative risk may not be statistically significant, it is consistent, and therefore probably real.* The difference in risk may be explained by considering the effect of oral contraceptive use. In most studies, the risk with oral contraceptive use is about half that with nonuse of any contraception.† Thus, in control populations in which there is a high proportion of oral contraceptive use, the overall risk of PID is lowered; in comparison, the relative risk of IUD use is elevated.

Many factors influence the apparent relationship of contraception to PID. First, PID is difficult to diagnose accurately on clinical grounds alone. One study showed the clinical diagnosis of PID to be confirmed laparoscopically for only 65% of the patients, while in another 15% of visually verified cases, the diagnosis of PID was not even considered prior to the procedure.[23] These misdiagnoses are probably of a random nature, and therefore, although they influence the PID rates found in the studies, their effect brings the relative risk closer to unity.[24] This epidemiologic premise was borne out by Faulkner and Ory[12] who found that a stricter case definition for PID raised the relative risk associated with IUD use.

Another aspect of the diagnostic dilemma is the relevance of organisms cultured from the cervix of women under study. PID is a disease that can be initiated by any of several organisms.[25] Cervical cultures of women with PID usually yield gonococcal organisms about 33% to 51% of the time,[26] but many other organisms can be isolated. Additionally, the spectrum and pro-

*In fact, three studies have confidence intervals which overlap at 1, indicating that the relative risks in those studies are not significantly different from 1. Thus, it may be that further work comparing IUD use to nonuse of any contraception eventually will show that IUD use raises the risk of PID only minimally.

†Four out of six studies have 95% confidence intervals which overlap at 1. However, as with studies of IUD use, the consistency of the results (i.e., relative risks of less than 1) supports the finding of lowered risk with oral contraceptive use.

portions of organisms reflect the prevalence of the organisms in a given population at a particular time and not an expected or average distribution. Thus, a diagnosis of PID should be considered whether or not a gonorrhea culture is positive. Studies in two countries have found no statistically significant difference in the percentage of IUD users and nonusers with PID from whom gonococci could be cultures,[4, 13] although one study[19] showed IUD users to have gonorrhea less frequently than nonusers. Another study[10] implicated IUD use in gonococcal PID. That same study[10] found a significantly lower prevalence of gonococcal PID for users of oral contraceptives despite a similar incidence of positive gonorrhea cultures in both groups. Eschenbach and co-workers [13] also found that oral contraceptive use may lower the risk of developing acute PID in women who have positive gonorrhea cultures.

Age and parity of women interested in fertility control obviously are related to the choice of contraception and have been investigated for their relation to PID. PID has been shown to be more prevalent in women less than 30 years of age.[2, 12, 27, 28] Using the Swedish data standardized for parity, Ory[1] found that the relative risk for IUD users remains higher for young women.

Parity has been addressed recently in four studies. Data collected by Weström and colleagues[4] yielded an age-standardized relative risk of 6.8 for nulliparous IUD users vs. 1.7 for multiparous IUD users, a statistically significant difference. However, exclusions from the control group for this study included a large proportion of nulliparous young women which could, in part, account for the tremendously increased risk for that group.[1] The Seattle study[13] showed nulliparous women using IUDs had a relative risk of 3.3 compared with non-IUD users, while previously pregnant women (parity not specified) had a relative risk of 2.1. The third study by Osser and his group,[28] based on age-standardized PID rates, did not confirm differing rates based on parity. The most recent study, also by Osser and associates,[19] showed no statistically significant difference in age-standardized relative risk of PID for parous and nulliparous women (2.6 and 1.5, respectively). Current data are inconclusive on this issue; however, parity appears to be less important than was thought earlier.

Further study on the effects of parity is needed, particularly because of the social significance of such findings. If PID does have long-term sequelae that affect the ability to bear children, and if IUD use carries a particularly high risk of developing PID for young nulliparous women, the recommendations and prescribing practices for contraceptives should reflect this finding. Conversely, if the hormonal contraceptives tend to lower the risk of PID, particularly for this high-risk group, these contraceptives may have added value in countries where infertility subsequent to PID or sexually transmitted diseases is a problem.

Socioeconomic status has been implicated as a risk factor for PID, and most of the epidemiologic studies control for this factor in some way. Eschenbach and associates[13] have evaluated the role of socioeconomic status in PID and found an estimated PID incidence of 4.1% among indigent IUD users and 1.3% among high-income women. They also found that the relative risk for IUD users was highest among the wealthier population and lowest among the indigent population. There are many possible explanations for this difference, including artifact based on a higher proportion of oral contraceptive users in the wealthier population. However, it may indicate that factors more directly correlated with socioeconomic status influence the PID rate more than does use of the IUD. Various authors have thought these factors to include use of or access to health care, general concern about health issues, differences in sexual habits, number of sexual partners (both male and female), general health status, and prevalence of sexually transmitted diseases in the population.

Ascent of microbes through the uterus and into the fallopian tubes is the probable origin of PID. Consequently, the fate of microbes introduced into the uterus during IUD insertion has attracted interest. Studies have shown that insertion of an IUD through the cervix introduces bacteria into the uterus; however, 1 month later no bacteria were found on endometrial culture.[21] More recently, evidence has shown that the uterus containing an IUD with a tail also contains bacteria similar to the vaginal flora, while the uterus without an IUD or with a tailless IUD contains no bacteria.[29] Studies by the same authors have shown that bacterial growth can occur in vitro on the cervical mucus adherent to the IUD tail.[29]

Several investigators have reported histologically confirmed sterile inflammation in the endometrium[21] and in the tubes[22] subsequent to IUD insertion. This inflammatory process has been proposed as the facilitator for ascending genital infection. Presumably, alterations in the inflamed tissues make clearance of invading bacteria more difficult and less complete. Also, perhaps because of mechanical damage by the IUD to the endometrial epithelium, bacterial access to the endometrial stroma is more direct.[21] Thus, when microbes are introduced intermittently into the uterus during menses or coitus,[25] they are more likely to cause infection. Because of the in vitro gonococcidal effect of copper, several investigators have attempted to demonstrate that copper-containing IUDs lower the risk of gonococcal infection in vivo. This, however, has not been found.[1]

The infective mechanism described is supported by the fact that PID predisposes to repeat PID, irrespective of gonococcal or nongonococcal etiology of the first attack.[25] Women who have had one episode of PID have a two- to threefold increase in the risk of having another episode compared to women who have never had PID. In repeat PID, there is a 50% increase in the proportion of nongonococcal cases over that in first-episode PID.[25] Thus, the distribution of causative agents in repeat PID more closely approximates the distribution in the general population. These findings point toward a residual process or change in the genital tract causing a decrease in the ability of the system to clear organisms or fight infection. Since the inflammatory process initiated by the IUD is similar to the inflammatory process initiated by infection,[22] one might assume that the inflammation per se is a predisposing factor for PID.

The mechanism of the protective effect of oral contraceptives against PID is speculative. Eschenbach[25] has postulated that the progesterone effect of increasing the density of cervical mucus may be responsible for inhibiting bacterial entry into the uterus. Weström and colleagues[4] have suggested that changes in uterine muscular activity may decrease propulsion of bacteria upward into the tubes, and decreased bleeding would reduce the availability of the culture medium for bacteria. The well known effect of steroids of decreasing the inflammatory response of the body in other situations also may play a part. Thus, it is physiologically plausible for the oral contraceptives to provide a lessened risk of PID.

Other contraceptive methods such as condoms and diaphragms with spermicidal chemicals apparently are associated with lower PID risks than the IUD[2] and are associated with a decreased prevalence of gonorrhea.[10] Several studies have found some in vitro activity of the spermicides against genital pathogens,[30-32] but no systematic study has compared effectively the risk of PID while using these agents to the risk associated with other contraceptives.

International implications

Despite methodologic difficulties, the available data clearly show an increased risk of PID with IUD use and a decreased risk of PID with oral contraceptive use. But a relative risk of 1.5 to 4 or of 0.5 does not convey the importance of these changes in risk in any given population. Concerns have been voiced over the health effects of various contraceptives, particularly for women in the developing countries. To address this issue, we have developed estimates of the strength of the PID-IUD relationship in the developing world. Consequent to the decrease in PID with oral contraceptive use, we also have estimated the decrease in PID resulting from use of oral contraceptives in developing countries.

Our methods are described elsewhere in detail.[33] Briefly, we estimated maximum and minimum numbers of women exposed to the IUD and to oral contraceptives in the underdeveloped countries, by region. We have excluded China, Britain, Canada, the United States, and Western Europe. Estimates of numbers of acute PID cases were based on the numbers of women using each method and on a conglomerate estimate of contraceptive method–specific rates of PID.

We estimated the total population of women aged 15 to 44 years to be 471.7 million in developing countries in 1978. The range of estimated IUD-associated cases of PID is 0.3 to 0.8 million, and the range of cases averted by use of the oral contraceptives is estimated to be 2.0 to 2.9 million cases.

Clearly, each contraceptive method has advantages and disadvantages. Our review and estimates indicate that one disadvantage of the IUD is an increase in PID associated with its use.* Conversely, an advantage of the oral contraceptives is the decrease in PID associated with their use. But PID is only one factor to consider; it is not the only factor. A reasonable approach would be to use these agents in age groups that have the most to gain (and, conversely, the least to lose) with each method. For instance, use of oral contraceptives might be encouraged in young women who wish to retain their fertility and to have relatively absolute control over the occurrence of a pregnancy; use of the IUD may be encouraged for women who have already completed their families and are not so concerned about, or possibly would welcome, the subfertility that might be a consequence of IUD-attributable PID, should it occur.

Data on the risks and benefits of various contraceptive methods have begun to clarify some of the issues. However, most of the research so far has been done in developed countries, and, consequently, there is a great gap in our knowledge of how these agents affect the populations of the developing world, where they also are used. Data on mortality, acute morbidity, and long-term sequelae are missing and necessary. Until such time as they are available, we must assume that the basic relationships of each contraceptive method to health are at least consistent in direction, if not in magnitude, anywhere in the world.

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*A related issue in the developing countries is whether to use individually prepackaged, sterile IUD and inserter units, or the considerably less-expensive bulk-packaged units, which must be sterilized at the time of use. Recently, there has been evidence from a study conducted in five developing countries[34] that the overall net rate of removal of IUDs because of PID is essentially the same for either type of packaging.

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Intrauterine device use and the risk of pelvic inflammatory disease

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A number of studies have shown an association between use of the intrauterine device (IUD) and the development of pelvic inflammatory disease. In the nine studies reviewed, the estimated risk varied between 1.6 and 9.3. A number of interacting variables are operant, in addition to IUD use. Further, the use of nonpermanent forms of contraception other than the IUD may exert a protective effect against the development of PID. (AM. J. OBSTET. GYNECOL. 138:861, 1980.)

THE POSSIBLE relationship between the use of an intrauterine contraceptive device (IUD) and the development of pelvic inflammatory disease has been an area of concern and research for over a decade. Starting with the report by Scott[1] on critical illnesses and deaths associated with the IUD and the review by Wright and Laemmle[2] concerning IUD use and pelvic infection, a number of studies have suggested that there is an association between IUD use and PID.

From a public health standpoint, this is a subject of some importance. For example, although there appeared to be a decline in rates of outpatient visits for salpingitis and PID from a peak in the early 1970s, the estimated rate in the United States remained at 2,500 visits per 100,000 female population aged 10 to 50 years in 1977.[3] In addition, other estimates indicate that between 1% and 2% of women discharged from hospitals in the United States have PID or salpingitis. Therefore, since reported studies indicate estimated relative risks of PID as high as 9.3 for IUD users,[4] these women could conceivably be at high risk for morbidity. This article will review some of the pertinent epidemiologic literature and also will discuss some of the problems in evaluating such studies.

Relevant epidemiologic literature

Table I lists nine selected studies that have shown an association between IUD use and PID.

The study by Wright and Laemmle[2] consisted of estimating the rate of acute PID in a predominately indigent black population, including estimates for substrata of contraceptive users. They showed that a febrile episode of PID occurred in 6.6% of IUD users as compared to 1.6% of nonusers of this form of contraception.

Targum and Wright[4] evaluated patients presenting with their first episode of PID and compared them to a control group consisting of women presenting to a general medical clinic and mothers of children seen in a pediatric emergency room. The 50 cases were matched to the 100 controls by age, current marital status, and interval since last pregnancy. In this study, 80% of the patients were hospitalized. In addition, one third of the case subjects wearing IUDs reported use of the Majzlin spring.

Lippes[5] compared the IUD usage rate of 91 women hospitalized at Buffalo General Hospital with PID in 1973 to that of 38 women with appendicitis. Fourteen percent of the PID patients as opposed to 8% of women with appendicitis were wearing an IUD on admission. Other potential variables affecting the occurrence of PID were not reported in this study.

Weström and co-workers[6] compared IUD usage patterns in 515 patients with laparoscopically verified acute salpingitis vs. 741 sexually active women from the same regions matched to the salpingitis group by date of birth, domicile, and socioeconomic status. In this study, the relative risk of salpingitis for IUD users compared to nonusers was 3.1 for all women, 6.9 for nulligravid patients, and 1.7 for previously pregnant women. About 40% of the originally selected control group were excluded for various reasons. Of significance was the exclusion of 301 women owing to lack of contraceptive use—a finding felt to be indicative possibly of lack of sexual activity. Also of interest was the finding of a rate of oral contraceptive use in the control subjects 250% higher than that in salpingitis patients.

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Table I. Selected studies indicating an association between IUD use and PID

Study	*Country*	*Design*	*Estimated risk of PID for IUD users*
Wright and Laemmle (1968)	U.S.A.	Record analysis	4.1
Targum and Wright (1974)	U.S.A.	Case-control	9.3
Lippes (1975)	U.S.A.	Case-control	1.9
Weström et al. (1976)	Sweden	Case-control	3.1
Faulkner and Ory (1976)	U.S.A.	Case-control	5.1
Vessey et al. (1976)	Great Britain	Prospective cohort	3.5
Eschenbach et al. (1977)	U.S.A.	Case-control	4.4
Kaufman et al. (1980)	U.S.A.	Case-control	6.5
The Women's Health Study[11]	U.S.A.	Case-control	1.6

Faulkner and Ory[7] compared 50 patients with PID and fever of at least 38° C and 100 patients with PID without fever to 200 patients presenting to the same emergency room who did not have an abdominal or gynecologic complaint. The women studied were from a predominately black indigent population in Atlanta, Georgia. IUD users were found to be five times more likely to develop febrile PID compared to nonusers. The risk did not change significantly when standardized for race, education, marital status, past diagnosis of PID, number of pregnancies, frequency of sexual intercourse, and number of partners. Controls were found to utilize forms of contraception other than the IUD more frequently than the febrile PID patients.

In cooperation with the Family Planning Association of Great Britain, Vessey and co-workers[8] carried out a prospective cohort study of 17,000 married white women, aged 25 to 39 years, currently using contraceptives, for 56,000 woman-years of observation. They determined a rate of first-episode hospitalization with salpingitis, PID, or endometritis of 0.83 per 1,000 woman-years. IUD users were found to be at 350% increased risk of hospitalization for these conditions compared to nonusers. This finding was not due to differences in parity, age, or social class.

Eschenbach, Harnisch, and Holmes[9] carried out a matched pair case-control study of 173 women presenting with PID and 173 controls, divided between women who presented at a venereal disease clinic or at a gynecology clinic. Matching was carried out for age, race, marital status, presence or absence of gonorrhea, parity, and number of sexual partners. The overall estimated relative risk of PID for IUD users was 4.4, with risks of 9.0 for nulligravid users and 3.4 for previously pregnant users. Thirty-two percent of the case group had experienced episodes of PID prior to inclusion in the study and 29% of the case group required hospitalization for the disorder.

Kaufman and co-workers[10] compared IUD usage patterns between 44 women hospitalized with a first episode of PID and 259 hospital control subjects. Excluded from the study were women experiencing pregnancy within 6 months of interview, postmenopausal women, and women who were not utilizing either the IUD or oral contraceptives. The age-adjusted relative risk of PID for IUD users was 6.5 and this risk persisted even when other factors such as parity were taken into account.

The Women's Health Study[11] was a multicenter, case-control study consisting of 1,457 women hospitalized with PID compared to 3,453 women hospitalized for nongynecologic surgical or medical conditions. Adjustment procedures were utilized in order to take into account differences in age, race, parity, frequency of sexual intercourse, and number of partners. For all those currently using IUDs the relative risk of PID was 1.6 but rose to 2.1 for patients experiencing a first episode of PID. The study demonstrated an increased association for women less than 25 years of age and for nonblack women, but there were no differences in risk between nulligravid and gravid women utilizing this form of contraception. Recency of insertion or reinsertion of an IUD was associated with increased risk but total duration of use was not. The study also showed that the relative risk of PID is higher when one compares IUD users to users of other contraceptives, as opposed to nonusers of contraceptives. Finally, the relative risk of PID for users of nonpermanent forms of contraception other than the IUD was less than 1—a finding suggestive of a protective effect.

Discussion

Defining PID is a major problem in evaluating the available literature. The term "pelvic inflammatory disease" is imprecise though in most usage it implies salpingitis. Furthermore, the symptoms and signs are not specific to the disorder and a variety of organisms including gonorrhea may be causative. In the study of Jacobson and Weström,[12] 905 women with signs and symptoms of PID underwent laparoscopy by the investigators. Although the diagnosis was confirmed in two thirds of the cases, one third of the patients did not have evidence of PID. Thus, a proportion of patients with suspected PID in the various studies may not, in fact, have had the disorder. Only one of the studies reviewed required visual confirmation of the PID diagnosis[6] although another did examine risks according to a scale of diagnostic certainty which required visualization for the "most certain" group.[11] However, in this latter study, the risk of PID for IUD users was slightly higher in the "less certain" groups than in the "most certain" group.

There also may be significant differences in the strengths of associations of factors depending upon whether or not one is dealing with first-episode acute PID or chronic forms of the disease. For example, patients with a past history of PID may not utilize the IUD as frequently owing to its being relatively contraindicated in such cases, yet prior IUD use may have been related to their contracting PID initially. Four of the studies reviewed[4, 8, 10, 11] dealt exclusively with or examined separately first-episode PID while the remaining studies did not.

Another important problem faced by investigators is the complex interrelationship of a number of variables, in addition to IUD usage, to this disease process. For example, number of sexual partners, frequency of sexual intercourse, and age all have been shown to affect the strength of association between IUD use and PID.[11] Also, nongonococcal forms of PID have been shown to occur more frequently among IUD users than gonococcal forms.[9] Therefore, in evaluating the various IUD and PID studies, one must take these other variables into account.

An important point being brought out by many of these studies is the suggestion that the use of nonpermanent contraception other than the IUD appears to convey a protective effect against the development of PID.[4, 6–9, 11] This is an important effect to recognize not only from its potentially beneficial aspects but also since some of the increased risk of PID reported for IUD users may be attributable partially to these reduced risks. For example, the risk reported in the study by Kaufman and associates[10] may have been lessened if sexually active non-contraceptive users had been included in the study. In any event, this phenomenon deserves further attention in order both to determine the mechanisms involved and to make users of non-IUD forms of contraception aware of this additional benefit.

Taking into account potential methodologic problems, variation in populations studied, and differing countries, the studies reviewed are consistent in showing an association between IUD use and PID. Thus, women wishing to utilize this form of contraception must be apprised of this risk so that they can make an informed judgment regarding the balance of risk and benefit for them.

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Risk of pelvic inflammatory disease among users of intrauterine devices, irrespective of previous pregnancy*

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The use of an intrauterine device (IUD) in 690 patients hospitalized for acute salpingitis (pelvic inflammatory disease) was compared with IUD use in a sexually active age-matched control group. Two hundred and twenty (31.9%) of the patients and 114 (16.5%) of the controls were wearing IUDs. Thus, the relative risk of developing acute salpingitis was 2 among IUD users. No significant difference was found in IUD use in the two groups between nulligravid women and women who had been pregnant. It was shown furthermore that the use of oral contraceptives decreases the risk of salpingitis by two thirds. A lower frequency of gonorrhea among PID patients who were IUD users was revealed also. (AM. J. OBSTET. GYNECOL. 138:864, 1980.)

ONE OF THE MOST serious complications of the use of an intrauterine device (IUD) is pelvic inflammatory disease. It has been estimated that two thirds of critical illnesses in connection with the use of IUD are infections.[1] Kahn and Tyler[2] found that one third of the IUD users who entered the hospital did so because of an infection. In the last few years the use of copper-medicated IUDs has increased. In Sweden copper IUDs (mainly TCu-200) are now almost the only type used.

During the last 5 years several investigators,[3-6] but not all,[7] have reported a large number of IUD wearers among patients with PID. At present it generally is accepted that the use of an IUD, irrespective of type, increases the risk of acute salpingitis. It still is debatable whether IUD users who have never been pregnant are more prone to develop IUD-related salpingitis than those who have been pregnant. Weström and colleagues[6] found the use of an IUD to increase the risk of salpingitis in nulligravid women sevenfold, compared with twofold in those who had been pregnant. The difference had not been shown previously in a controlled study. Ory,[8] however, questioned the importance of pregnancy. In an earlier prospective follow-up study,[9] we found no difference between parous and nulliparous women; however, that study was done without any control group.

In the light of the above lack of unanimity we tried to find out whether salpingitis is more common among IUD users than in a sexually active age-matched control group; and if so, whether women who previously have been pregnant differ in this respect from those who have not.

Patients and methods

The study covered a 3 year period (1973 to 1975), during which 735 patients with acute salpingitis were admitted to the Department of Obstetrics and Gynecology in the General Hospital of Malmö. This hospital serves about 250,000 inhabitants of Malmö and is the only gynecologic unit in the town. In the majority (over 86%) of patients the diagnosis was verified by laparoscopy. We could not trace the records of 15 of the patients. This left 720 patients for the study. All relevant gynecologic and obstetric data were extracted from the records (age, previous pregnancies, methods of contraception, laparoscopy, result of culture for gonorrhea).

For each patient, a woman born on the same day and year was selected from the Public Register of Births

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**Previously published in part as: Osser, S., Liedholm, P., Gullberg, B., and Sjöberg, N-O.: Risk of pelvic inflammatory disease among intrauterine-device users irrespective of previous pregnancy, Lancet 1:386, 1980.*

and served as a control. A questionnaire was sent by mail to each control. The questions concerned data about the controls during the month the matched patient had been hospitalized for salpingitis. Each woman was asked whether she had been pregnant that month; whether she had been pregnant any time before that month; whether she had been healthy, ill, or hospitalized that month; whether she had used any method of contraception that month, and if so, which method; how many sexual partners she had; and how often she had had intercourse monthly during that period. The purpose of these questions was to exclude any women not sexually active during that specific month. The first returned questionnaire not eliminated using the following exclusion criteria was taken as a matched control: women whose addresses could not be traced, pregnant women, ill or hospitalized women, and women who had no sexual partner or intercourse during the period in question. After this elimination procedure 690 (95.8%) of the 720 patients had one control each.

The remaining 30 women with salpingitis but with no control did not deviate from the other cases in any other respect and were excluded from further study analysis. The following techniques were used: Cochran's test for multidimensional contingency table, log-linear test, and Mantel-Haenszels estimate of the mean odds ratio.[10, 11] All tests were two-tailed.

Results

The ages of the patients ranged between 14 and 49 years. The mean age was 24 years and as many as 80.7% of the entire series were between 16 and 30 years of age. Two hundred and ninety-one (42.2%) of the patients had not been pregnant previously and 399 (57.8%) had been pregnant. The corresponding figures in the control group were 266 (38.5%) and 424 (61.5%).

Table I lists the contraceptive methods used. Only a minority used plastic IUDS, 13 (5.9%) in the PID group and 11 (10.0%) in the control group. Table II lists the age distribution and frequency of the IUD users among all the patients who had and had not been pregnant previously.

The interval between insertion of the IUD and the onset of salpingitis in 211 of the 220 patients (not known in the remaining nine) is shown in Table III. The mean interval was 11 months in the women who had been pregnant previously, 6 months in those who had not, and 10 months in all subjects. This difference between the groups is, however, not significant when adjusted for age. The cultures were positive for gonorrhea in 154 (22.3%) of the 690 patients but in only 32 (14.5%) of those (220) with PID and using an IUD. The difference was significant ($p < 0.002$). Thus among the patients with gonococcal PID there were 20.8% IUD users compared to 35.1% users among the 536 nongonococcal PID cases.

Table I. Frequency of contraceptive methods among patients and controls

	PID	*Controls*
IUD	220 (31.9%)	114 (16.5%)
Oral contraceptive	159 (23.0%)	324 (47.0%)
Other or no method	311 (45.1%)	252 (36.5%)
Total	690	690

Among the 154 women with gonococcal salpingitis, 20.8% used an IUD while 35.1% of the 536 women with nongonococcal salpingitis used an IUD. Of the 690 patients, 594 (86.1%) underwent laparoscopy. No difference in laparoscopic findings or symptoms was found between users and nonusers of IUDs.

All the controls were sexually active and the frequency of intercourse was, on the average, nine times monthly. Eighty-nine percent had a single sexual partner. Among the controls no difference in sexual activity was noted between the women who had never been pregnant and those who had, or between those who were wearing an IUD and those who were not.

Discussion

The frequency of IUD users among PID patients in different controlled studies ranged from 14% to 48%.[3-7, 12] In our study it was 31.9% compared with 16.5% of the controls. The relative risk of IUD users developing salpingitis in this study was 2.1, which is highly significant ($p < 0.0001$; 95% confidence interval is 1.6 to 2.7). According to Weström and associates[6] and Eschenbach and Soderstrom[13] the relative risk of PID in IUD users is greater for nulligravid than for previously pregnant women. It has been suggested by Ory[8] that part of the difference found in the study by Weström and co-workers can be explained by a biased selection of their controls, i.e., dropouts in young age groups, etc. The present study was performed in the same region of Sweden, where the population is comparable in regard to socioeconomic status. In our study we found no significant difference in the frequency of salpingitis among the women who had been pregnant previously and nulligravid women. The risk for the former was 2.6 ($p < 0.001$) and for the latter 1.6 ($p < 0.02$). The difference between 1.6 and 2.6 is not significant. Similar results have been reported by Nygren and Nielsen.[14]

Unlike Weström and associates[6] we asked the control

Table II. IUD use among previously pregnant and nulligravid women with PID and controls, by age

Age group (yr)	*PID*				*Controls*			
	Parous		*Nulligravid*		*Parous*		*Nulligravid*	
	IUD	*No IUD*	*IUD*	*No IUD*	*IUD*	*No IUD*	*IUD*	*No IUD*
<16	1	2	2	6	0	1	2	8
16-20	20	54	26	114	3	14	21	176
21-25	53	67	25	74	20	57	25	117
26-30	44	53	3	24	17	67	5	35
31-35	31	33	1	10	10	39	2	24
>35	14	27	0	6	9	29	0	9
Total	163	236	57	234	59	207	55	369
% With IUD	40.9%		19.6%		22.2%		13.0%	

Table III. Interval between insertion and onset of salpingitis in previously pregnant and nulligravid women, by months

Mo	*Parous*	*Nulligravid*	*All patients*
<1	42 (25.8%)	26 (45.6%)	68 (30.9%)
2-6	33 (20.2%)	9 (15.8%)	42 (19.1%)
7-12	35 (21.4%)	12 (21.1%)	47 (21.4%)
>12	45 (27.6%)	9 (15.8%)	54 (24.5%)
Total	155	56	211

women whether they were sexually active, i.e., we neither considered the mere use of a contraceptive as proof of sexual activity, nor did we exclude women not using contraceptives. It is well known that in every population there are a significant number of women who are sexually active and do not use any contraceptive method. In the present study every patient had her own control. Thus, the age distribution of the two groups was identical; in this respect our sample was unbiased, a point that Ory[8] finds very important.

In many hospitals the diagnosis of PID is made on clinical grounds, and is therefore less certain. In this study the diagnosis usually was confirmed (86.1%) by laparoscopy. A complete statistical analysis concerning the frequency and distribution of salpingitis not verified by laparoscopy resulted in the same IUD frequency and risk figures in the different groups.

Women never previously pregnant were prone to develop salpingitis within a month after insertion of the IUD ($p < 0.04$; age stratified). This might be explained by mechanical trauma to the narrower cervical canal during insertion in these women. Oral contraceptives were used by 23.0% of the patients and by 47.0% of the controls. This difference is highly significant ($p < 0.0001$), and is consistent with the finding by Weström and colleagues.[6] This might be due to the hormones of oral contraceptives increasing the viscosity of the cervical mucus. Perhaps the shortening of the menstrual bleeding is also a contributory factor. In our study oral contraceptives reduced the relative risk of developing salpingitis by two thirds.

In vitro studies have suggested that copper IUDs can offer protection against gonorrheal infection,[15] but this could not be demonstrated by Weström and his associates.[6] Our study revealed a significantly lower frequency of gonorrhea among PID patients who were IUD users than among nonusers ($p < 0.002$), possibly because the IUD was medicated with copper.

On the other hand, the greater frequency of gonorrhea among non-IUD patients possibly could be explained by the hypothesis that both gonococcal infection and IUD use are relatively independent risk factors for PID.

Several explanations have been offered for the increased frequency of PID among IUD users. Thus, in connection with insertion of the IUD, bacteria are introduced into the uterine cavity despite aseptic precautions.[16] Menorrhagia and intermenstrual bleedings are common among IUD users and the bleeding changes the vaginal and cervical milieu, facilitating ascending infections. An IUD in utero also may alter the defense mechanisms that normally remove bacteria. The main conclusion of this study is that the use of an IUD doubles the risk of acute salpingitis (compared with the risk in a sexually active age-matched control group). Although the risk is increased in the woman who has never been pregnant, the difference in relative risk is not significant. We think that the risk for nulligravid women has been exaggerated during the last few years. But salpingitis may, of course, imply a greater risk of psychological and practical problems in women who have never been pregnant but who wish to retain their fertility.

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Pattern of acute pelvic inflammatory disease in abortion-related admissions

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We evaluated 3,066 consecutive women admitted during 1 year to two major hospitals of Kuala Lumpur and the adjacent urban area of Malaysia. Indicators of acute pelvic inflammatory disease were more common among patients with induced abortions. PID was thought to be a major contributor to the higher costs associated with management of patients with induced abortions. (AM. J. OBSTET. GYNECOL. 138:868, 1980.)

ACUTE PELVIC inflammatory disease is a serious consequence of abortion. This brief report on clinical aspects of women hospitalized for abortion focuses on those features related to PID.

We studied women hospitalized at the Government General Hospital (Hospital Besar) of Kuala Lumpur (Bandarya Kuala Lumpur) and the University Hospital, University of Malaya, of the adjoining municipality of Petaling Jaya. The estimated population of the Kuala Lumpur–Petaling Jaya urban area is approximately 1.4 million. The two study hospitals are the only major public hospitals with obstetric and gynecologic units which serve this population. A few small private hospitals and nursing homes/clinics also care for some women with abortion-related gynecologic illnesses.

Methods

This study is a small component of the multicenter Prospective Hospital-Based Study on Illegally Induced Abortion sponsored and funded by the World Health Organization. Other centers which participated in this study were located in Ankara, Turkey, Caracas, Venezuela, and Lagos, Nigeria. All four centers used standardized, although not identical, protocols, questionnaires, and data collection instruments.

In Malaysia, all women admitted to the two study hospitals during the year October, 1976, to September, 1977, were evaluated for possible abortion. All patients identified as having an abortion-related hospital admission during this year became part of the study group. These patients were interviewed by the study team and their records were abstracted. Their routine care, however, was provided by the normal hospital staff.

Standard definitions of abortion type were used at both hospitals and were based on the patient histories. Patients were considered to have had manipulation after the abortion if they described or underwent a second pelvic procedure following the initial abortion activity. Any patient with an oral temperature of 38° C recorded during the hospital stay was considered febrile. If adnexal tenderness was recorded by an experienced consultant, the patient was considered to have tenderness. Patients' estimates of blood loss prior to hospitalization and the hospital estimates of blood loss

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Table I. Abortion types in the two study hospitals—abortion-related hospitalizations, Malaysia, 1976-77

	University Hospital		*General Hospital*		*Total*	
Types of abortion	*No.*	*%*	*No.*	*%*	*No.*	*%*
Therapeutic abortion with sterilization	105	9.5	5	0.3	110	3.6
Therapeutic abortion only	11	1.0	17	0.9	28	0.9
Menstrual regulation	259	23.7	2	0.1	261	8.5
Induced abortion (certain)	72	6.6	103	5.2	175	5.7
Induced abortion (massage unsuccessful)	17	1.6	46	2.3	63	2.1
Induced abortion (probable)	80	7.3	362	18.4	442	14.4
Induced abortion (possible)	38	3.5	243	12.3	281	9.2
Spontaneous abortion	374	34.2	821	41.6	1,195	38.9
Unclassifiable	139	12.7	373	18.9	512	16.7
Total	1,095	100	1,972	100	3,067	100

Table II. Admission findings related to abortion types—abortion-related hospitalizations, Malaysia, 1976-77

	Manipulation history (no.)		*Foreign body present (no.)*		*Fever 38° C (no.)*		*Adnexal tenderness (no.)*		*Blood loss history (no.)*	
Abortion types	*Yes*	*No*	*Yes*	*No*	*Yes*	*No*	*Yes*	*No*	*Excessive*	*No*
Induced (n = 238)	28	210	9	229	82	156	43	195	14	224
Therapeutic (n = 138)	1	137	—	138	—	138	—	138	2	136
Menstrual regulation (n = 261)	1	260	—	261	—	261	—	261	2	259
Spontaneous (n = 1,195)	5	1,190	—	1,195	24	1,171	14	1,181	11	1,181
Total no. (%)	35 (1.9)		9 (0.5)		106 (5.8)		57 (3.1)			

Table III. Blood transfusion use related to abortion type—abortion-related hospitalizations, Malaysia, 1976-77

	Yes		*No*		
Types of abortion	*No.*	*%*	*No.*	*%*	*Total*
Induced	34	14.3	204	85.7	238
Therapeutic	6	4.3	132	95.7	138
Menstrual regulation	1	0.4	260	99.6	261
Spontaneous	83	7.0	1,112	93.0	1,195
Total no. (%)	124 (6.8)		1,708 (93.2)		1,832 (100)

were combined to estimate total blood loss. Hospital records of transfusions and antimicrobial use were used to document these treatments.

Costs for antimicrobials were based on the actual costs to the hospitals of the drugs administered. Other hospital costs include bed occupancy and surgical and anesthetic costs, and are based on hospital estimates of actual costs. All costs were converted to U. S. dollars for standardization.

Further data compilation and analysis are in progress.

Results

During the year of the study, 3,067 consecutive women were hospitalized in the two study hospitals for abortion-related illness. Of these patients, 1,095 (35.7%) women were admitted to the University Hospital and 1,972 (64.3%) to the General Hospital. Admissions for acute gynecologic illnesses were distributed similarly between these two hospitals; approximately one third were admitted to the University Hospital and two thirds to the General Hospital.

Although abortion types were classified similarly at both hospitals, the pattern was different for these two institutions (Table I). Both "therapeutic abortion with sterilization" and "menstrual regulation" were more common in the University Hospital (9.5% and 23.7%, respectively) than in the General Hospital (0.3% and 0.1%). Otherwise, the abortion types were similar.

Abortion type and selected clinical parameters. We identified four groups of well defined abortion types in order to better compare clinical parameters and health factors to these. These groups are: (1) induced abortion consisting of 238 cases, which include 175 cases of

Table IV. Antimicrobial use for 169 patients with induced abortion—abortion-related hospitalizations, Malaysia, 1976-77

Type of antibiotics	*No. of courses*	*% Antibiotic courses*	*% Respondents given particular antibiotics (n = 169)*
Ampicillin (Penbritin)	114	47.7	67.5
Penicillin	44	18.4	26.0
Streptomycin	43	18.0	25.4
Co-trimoxazole (Bactrim/Septrin)	23	9.6	13.6
Tetracycline (Achromycin)	6	2.5	3.6
Cephaloridine (Ceporin/Ceporex)	5	2.1	3.0
Kanamycin	2	0.8	1.2
Chloramphenicol (Chloromycetin)	1	0.4	0.6
Gentamicin	1	0.4	0.6
Total	239	100	141.5

Table V. Medication costs by abortion type—abortion-related hospitalizations, Malaysia, 1976-77

	Medication costs ($ U. S.)												
	None		*≤$20*		*$21-$50*		*$51-$80*		*$81-$110*		*$111-$140*		
Abortion types	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*Total no.*
Induced	12	5.0	146	60.6	71	29.5	9	3.7	1	0.4	2	0.8	241
Therapeutic	2	1.4	131	95.0	5	3.6	—		—		—		138
Menstrual regulation	42	16.1	219	83.9	—		—		—		—		261
Spontaneous	222	18.6	905	75.7	62	5.2	5	0.4	1	0.1	—		1,195
Total	278	15.1	1,401	76.4	138	7.5	14	0.8	2	0.1	2	0.1	1,835

"induced abortion (certain)" and 63 cases of "induced abortion (massage unsuccessful)"; (2) therapeutic abortion consisting of 138 cases, which include 110 cases of "therapeutic abortion with sterilization" and 28 cases of "therapeutic abortion only"; (3) menstrual regulation consisting of 261 cases; and (4) spontaneous abortion consisting of 1,195 cases.

These 1,832 cases were clear-cut abortion types. The remaining 1,234 cases were less clearly defined abortion types and will not be considered further in this report.

On admission, 11.8%of the induced-abortion group had evidence of manipulation; less than 1% of each of the other three groups had such evidence (Table II). Foreign bodies were present in nine patients (3.8%) of the induced group (Table II). No foreign bodies were found in the other three groups.

Eighty-two patients in the induced-abortion group (34.5%) had fever of 38° C or higher (Table II). In contrast, no patients in the therapeutic-abortion or menstrual-regulation groups had fever and only 14 (2%) in the spontaneous-abortion group had fever.

Adnexal tenderness, either unilateral or bilateral was identified in 18% (43 cases) of the induced-abortion group (Table III). In contrast, no patients had anexal tenderness in the therapeutic-abortion and menstrual-regulation groups; only 1.2% of the patients in the spontaneous-abortion group had adnexal tenderness.

Excessive blood loss (loss of more than 100 ml) was four to six times more common in the induced-abortion group (5.9%) when compared with the other three groups (Table II). Thus, 1.4% of the therapeutic-abortion, 0.8% of the menstrual-regulation, and 0.9% of the spontaneous-abortion groups had blood loss exceeding 100 ml.

Case management. Blood transfusions were used in 14.3% of the induced-abortion group, 7.0% of the spontaneous-abortion group, and 4.3% of the therapeutic-abortion group (Table III). Only one patient (0.4%) in the menstrual-regulation group received a transfusion.

Antimicrobials were administered for genitourinary infections to 169 induced-abortion patients, or 71% of this group. No other group received antimicrobial agents. These 169 patients received 239 courses of antimicrobials (Table IV). Ampicillin, the antibiotic used most commonly, was given to 67.7% of these patients and constituted 47.7% of all chemotherapy courses. A combination of penicillin and streptomycin, the next most popular regimen, constituted about 18% of all antibiotic courses and was used to treat nearly 26% of these patients. The co-trimoxazole type of combination

Table VI. Hospital costs (excluding antibiotic charges) related to abortion types—abortion-related hospitalizations, Malaysia, 1976-77

	Hospital costs, excluding antibiotic charges ($ U. S.)														
	None		*≤$100*		*$101-$200*		*$201-$300*		*$301-$400*		*$401-$500*		*≥$501*		
Abortion types	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*Total no.*
Induced	—		3	1.3	80	33.8	91	38.4	36	15.2	13	5.5	14	5.9	237
Therapeutic	—		3	2.4	96	76.2	5	4.0	14	11.1	1	0.8	7	5.6	126
Menstrual regulation	256	98.5	3	1.2	1	0.4	—		—		—		—		260
Spontaneous	1	0.1	29	2.4	608	50.9	288	24.1	162	13.6	46	3.9	60	5.0	1,194
Total	257	14.1	38	2.1	785	43.2	384	21.1	212	11.7	60	3.3	81	4.5	1,817

sulfonamide constituted nearly 9.6% of all antimicrobials administered and was given to 13.6% of the patients. Other antibiotics were used infrequently.

Hospitalization costs. For 34.4% of the induced-abortion group, medication costs exceeded $20 (Table V). Only 3.4% of the therapeutic-abortion group, 5.7% of the spontaneous-abortion group, and none of the menstrual-regulation group incurred medication costs of $21 or more.

When medication costs were excluded, hospitalization costs for the induced-abortion group still exceeded those for other groups (Table VI). Nearly 65% of induced abortions incurred cost exceeding $200. In contrast, only 46.6% of spontaneous abortions and 21.4% of therapeutic abortions incurred hospital costs exceeding $200. None of the menstrual-regulation treatments incurred such high costs.

Discussion

Over 3,000 abortion cases were identified in this study. Other patients who failed to develop illness after an abortion were not counted, and still others who received care elsewhere were not included. Thus, even where laws limit abortion practices, these are relatively common procedures.

Nearly one third of all the abortion-related admissions were patients with induced abortions (Table I). Such patients were more likely to have a febrile illness and adnexal tenderness. These signs probably are caused by acute PID in this population.

Patients with induced abortion were more likely to require blood transfusions, both to replace excessive blood loss and to combat bacteremic shock (Table III). This excessive blood loss probably is greatly underestimated in this group, since these patients, fearing legal and social consequences, delayed seeking treatment.

Cases of induced abortions incurred much higher medical costs than other cases. Such cost excess was attributed to antibiotic use and to longer hospitalizations. We ascribe both these factors to the higher PID risks in the group of patients who had induced abortions. Part of the relatively high hospitalization costs associated with spontaneous abortions were due merely to delays of at least 1 day between admission and surgical evacuation at one of the hospitals because anesthesia/theater facilities were lacking.

We were surprised that only 1.9% of these patients had evidence of manipulation. In prior studies in rural Malaysia, as many as 12.9% of all abortion cases had had manipulation.[1, 2] Most induced abortions performed in this study were done by Western-trained physicians. Since such personnel are not available to rural populations, a larger percentage may have manipulation after the abortion.

The excellent assistance of Mrs. Ivy Phang, University of Malaya, in preparation of this manuscript is gratefully acknowledged. We sincerely thank the World Health Organization Task Force on Illegal Abortion for their generous funding and support in undertaking this extensive multicenter hospital-based abortion study.

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Etiology of pelvic infections treated by the gynecologic service of the Kasturba Hospital, Delhi, India

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Selected etiologic and clinical characteristics of pelvic infections associated with gynecologic surgical procedures and other conditions were studied. During a 5 year period, 1,800 patients were admitted to Kasturba Hospital. Infections following sterilization by vaginal tubectomy were common while septic-induced abortion was the leading cause of maternal morbidity. Medical management was successful in most of the patients. (AM. J. OBSTET. GYNECOL. 138:872, 1980.)

PELVIC INFECTIONS are common problems encountered in gynecologic, infertility, family planning, postnatal, legal abortion, and sterilization clinics in India. These infections may be caused by specific pathologic microorganisms, such as *Neisseria gonorrhoeae* and *Mycobacterium tuberculosis,* or nonpathologic bacteria. Genital tuberculosis, though rare in Western countries, is still quite prevalent in India.[1, 2] Patients with this disease often have no signs or symptoms and often are diagnosed during routine evaluations or investigations for primary and secondary amenorrhea.

Depending on socioeconomic status, minor symptoms due to pelvic infections often are ignored by the patient and medical care is sought only when acute symptoms develop or when menstrual disorders or infertility occurs. In spite of sex education in schools, increased awareness of venereal diseases, and better methods of diagnosis and management, pelvic infections still are not being treated properly. As a result, they frequently progress to pelvic abscesses, adhesions, and chronic symptoms.

In the presence of liberalized abortion laws, increasing numbers of women are seeking legal abortions. Unless abortion procedures are performed in the first trimester in recognized clinics under hygienic conditions, pelvic infection will be a common occurrence, with subsequent secondary infertility, menstrual disorders, and lower abdominal pain.

This article reviews the general experience of the gynecologic service of the Kasturba Hospital with respect to selected etiologic and clinical aspects of pelvic infections.

Method

For all patients admitted to the gynecologic service of Kasturba Hospital, recording of a history and a complete physical examination, including a pelvic examination, are done routinely. In addition, the following tests are performed: (1) blood—hemoglobin, white cell differential count, erythrocyte sedimentation rate, VDRL,* and blood culture, when indicated; (2) urine—routine, microscopic, and culture, if necessary; (3) wet smear for *Trichomonas* and monilial infection; (4) Gram stain for gonococcal infection; and (5) high vaginal swab for culture and sensitivity.

All patients with pelvic infections present on admission or developing as a result of gynecologic interventions received medical therapy consisting of antibiotics. Penicillin, streptomycin, and chloramphenicol (in severely ill patients) were used initially until the results of culture and sensitivity testing were known. Some patients also received antiinflammatory medications such as cortisone. Pelvic diathermy occasionally was done in cases of chronic infection. Surgery was reserved for patients who failed to respond to medical therapy.

Diagnostic laparoscopy was performed when the diagnosis was in doubt, in case of unexplained infertility, for menstrual disorder, for primary and secondary amenorrhea, and in some patients complaining of pain without positive findings on pelvic examination.

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Table I. Pelvic infection (PI) rate in gynecologic patients sorted by surgical and nonsurgical causes

Causes of PI	*No. of patients*	*No. of PIs (%)*
Surgical interventions:		
1. Vaginal sterilization	1,718	167 (9.7)
2. Vaginal sterilization with medical termination of pregnancy	724	108 (15.0)
3. Laparoscopy sterilization	13,924	118 (0.85)
4. Laparoscopy sterilization with medical termination of pregnancy	2,194	41 (1.9)
5. Abdominal sterilization	3,388	100 (2.95)
6. Minilaparotomy	469	8 (1.7)
7. Minilaparotomy with medical termination of pregnancy	111	12 (10.8)
8. Culdoscopy	152	5 (3.3)
9. Medical termination of pregnancy	4,338	130 (0.3)
Subtotal	27,018	689 (2.6)
Nonsurgical interventions:		
10. Abortions including septic	14,769	317 (2.1)
11. Intrauterine device	106,580	405 (0.38)
12. Puerperium	NA*	233
13. Infertility	NA	156
Subtotal	NA	1,111

*Number not available.

Results

During the 5 year period from 1975 to 1979, 63,308 patients were admitted to the gynecologic service. Among these were 1,800 patients (2.8%) with pelvic infections. Their ages varied between 19 and 35 years and included both nulliparous and multiparous women. Table I summarizes the number and rate of pelvic infections (PIs) in women undergoing gynecologic surgery by type of surgical procedure. This table also includes the additional conditions associated with PI, i.e., abortions, insertion of intrauterine devices, puerperium, and infertility. A total of 27,018 women underwent a surgical procedure and pelvic infections developed in 689 (2.6%). Patients who underwent sterilization by vaginal tubectomy and minilaparotomy with abortion had the highest PI rate (9.7% and 10.8%, respectively).

Symptoms and signs in women with PI are presented in Table II. Lower abdominal pain is the most common symptom while vaginal discharge, tuboovarian masses, and pelvic cellulitis were the most common signs.

A total of 540 women (30%) underwent diagnostic laparoscopy. The findings are presented in Table III. Tubo-ovarian masses or hydrosalpinx associated with PI occurred in 27.9% of the women. Abdominal tuberculosis was diagnosed in 4.8%.

Medical therapy failed in 80 cases (4.4%). Total hysterectomies were necessary in only 10 women; the remainder responded to more conservative measures such as removal of tubo-ovarian masses, needle colpotomy, abscess drainage, and other repair procedures.

Table IV summarizes the cause of death among the 1,800 patients with PI. The mortality rate was 2.4% (43 women) with the majority (46.5%) succumbing to infection resulting from septic and induced abortions.

Table II. Predominant symptoms and signs among 1,800 women with pelvic infections

	No. of cases with a predominant symptom or sign
Symptoms	
Abdominal pain	360
Infertility, primary and secondary	130
Amenorrhea	64
Signs	
Tubo-ovarian mass, pelvic abscess, and cellulitis	400
Vaginal discharge	400
Fever	280
Bleeding per vagina	110
Severe anemia	30
Peritonitis with gastroenteritis	20
Bartholin abscess	5
Septicemia with renal failure	1

Table III. Diagnostic laparoscopy results, 540 cases

	Cases	*%*
Pelvic infection with tubo-ovarian mass or hydrosalpinx	130	27.9
Genital or abdominal tuberculosis	26	4.8
Other	384	67.4
Total	540	100

Table IV. Deaths due to pelvic infection

Specific cause	*Deaths*	*%*
Septic-induced abortion	20	46.5
Puerpural sepsis	12	27.9
Others	11	25.6
Total	43	100

Discussion

As our data demonstrate, pelvic infections are common in young women. Excluding PI following sterilization by tubectomy, many diagnoses of PI were made in women who were of low parity or in infertile women.

The most common infecting organisms were *Escherichia coli* and anaerobic bacteria. Isolation of *N. gonorrhoeae* was uncommon either because patients ignore their symptoms and do not attend our clinics or they seek treatment at nearby special venereal disease clinics.

In India, intrauterine devices (IUDs) are commonly used contraceptives. The incidence of PI following insertion was low (0.38%) compared to rates reported by Gulati and Kapoor[1] and Wilson and associates[3] (2.4% and 7.7%, respectively). Patients who underwent tubectomy following removal of their IUDs showed a higher incidence of PI.

Because of the high incidence of PI following sterilization by vaginal tubectomy, this procedure has virtually been abandoned in Kasturba Hospital.

In spite of liberalized abortion laws, septic-induced abortion is still common, and patients often are admitted in advanced moribund conditions resulting from widespread infection. Almost half of the deaths occurred in women with septic-induced abortions. This condition was seen most frequently in unmarried, young women in whom the abortion was performed in the second trimester by untrained persons.

Puerperal sepsis is common since many patients' babies are delivered at home under less-than-ideal conditions. These women seek care when their conditions become serious and death may result.

Medical therapy was successful in the vast majority of our cases, and we recommend surgery only for treatment failures. We urge complete treatment following adequate diagnostic evaluation to prevent the occurrence of resistant organisms. In addition, public education, personal hygiene, early medical care, and sex education in schools may be effective in reducing the incidence of pelvic infections.

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Pelvic infections following gynecologic procedures in Bangladesh

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Data were collected to establish the rate of pelvic infection following various gynecologic procedures, including postpartum intrauterine device (IUD) insertions, IUD insertion following menstrual regulation procedures, IUD insertion in women who were not recently pregnant, and induced and spontaneous abortions. The pelvic infection rate was only 1% in 97 women who underwent postpartum IUD insertions. The infection rate was related to the timing of IUD insertion and the type of IUD used. Finally, the pelvic infection rate was unacceptably high (10.3%) in women undergoing induced abortions vs. women having spontaneous abortions (1.1%). (AM. J. OBSTET. GYNECOL. 138:875, 1980.)

PELVIC INFECTIONS are common problems in developing countries. Many events, such as the insertion of an intrauterine contraceptive device (IUD), delivery of a baby, spontaneous or induced abortions, and gynecologic manipulations, may increase the risk of pelvic infection. Although the presence of an IUD may increase the risk of pelvic infection, the rate of infection may vary depending on the timing of IUD insertion in relation to other gynecologic events. In the event of an incomplete abortion, the probability of a subsequent pelvic infection may vary depending on whether the abortion was induced or spontaneous.

Data were analyzed from the Dacca Medical College Hospital Obstetrical Department and the Mohammadpur Model Clinic to evaluate the association between pelvic infections and two gynecologic events: (1) IUD insertion following (a) menstrual regulation procedures and (b) childbirth and (2) spontaneous and induced abortions.

Methods and material

The IUD insertion data from the Mohammadpur Model Clinic were collected as a part of a comparative study of Copper-T vs. Lippes loop IUDs, among two acceptor categories, women who had undergone a menstrual regulation procedure (post-MR) and women who were not recently pregnant (interval). For the interval category, the IUD was inserted within the first 7 days of a menstrual cycle, and for the post-MR category, the IUD was inserted within an hour of a menstrual regulation procedure in the Clinic. Women in both categories were given either a Lippes loop, size C, or a Copper-T 220C as indicated by predetermined random allocation cards.

Data were collected to determine the incidence of pelvic infection following postpartum IUD insertion in the Dacca Medical College Hospital. Our sample included 97 women who accepted the postpartum insertion of a Lippes loop, size D, with three–catgut suture extension. The IUD was inserted manually (without the help of any inserter) immediately after expulsion of the placenta following childbirth.

Data also were collected on incomplete abortions as part of a hospital abortion study. This study included 1,003 patients admitted to the Dacca Medical College Hospital for management of incomplete abortion, either induced or spontaneous.

All study data were recorded on the standard International Fertility Research Program (IFRP) forms. The data recording was monitored by local fertility research staff, and later the data were analyzed at IFRP in Research Triangle Park, North Carolina.

Results

Table I presents the incidence of pelvic infection following insertion of Copper-T and Lippes loop IUDs in post-MR and interval patients. The infection rate for the Copper-T IUD was somewhat higher than that for the Lippes loop IUD (approximately 1.7 times greater).

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Table I. Incidence of pelvic infection among post-MR and interval IUD acceptors with relative risk, 95% confidence interval

Timing of IUD insertion	Copper-T		Lippes loop		Total	
	N	*% Infected*	*N*	*% Infected*	*N*	*% Infected*
Post-MR	172	7.5 (13)	179	5.0 (9)	352	6.3
Interval	115	3.4 (4)	119	0.8 (1)	234	2.1
Relative risk	2.2		6.2		3.0	
95% Confidence interval	(0.0, 9.2)		(0.6, 7.7)		(1.0, 7.3)	
P	0.162		0.05		0.102	

Table II. Incidence of infection among induced and spontaneous abortion patients in Dacca Medical College Hospital

Type of abortion	*Abortion patients (N = 1,003)*		
	N	*No. infected*	*% Infected*
Induced	243	25	10.3
Spontaneous	760	8	1.1

However, this difference was not found to be statistically significant. On the other hand, the infection rate was considerably greater among post-MR patients compared to interval patients for both types of IUDs (7.5% vs. 3.4% among Copper-T acceptors and 5.0% vs. 0.8% among Lippes loop acceptors). Overall, the risk of pelvic infection for post-MR patients was threefold that for interval patients (95% confidence interval, 1.0 to 7.3). The difference in the infection rate between post-MR and interval cases among Lippes loop users was statistically significant (95% confidence interval, 0.6 to 7.7).

Of the 97 women who accepted the postpartum insertion of a Lippes loop, only one (1%) developed a pelvic infection. This infection rate is somewhat comparable to the infection rate among interval IUD users (2.1%).

Table II presents the incidence of pelvic infection among patients with induced and spontaneous abortions. Among women with induced abortions, 10.3% had pelvic infections compared to only 1.1% of the patients with spontaneous abortions.

Discussion

A higher rate of infection among post-MR IUD users apparently would suggest that women desiring a IUD for contraception following an MR procedure should postpone insertion of the IUD for a few weeks. However, this conclusion may not be valid given the possibility that the higher infection rate among post-MR IUD users could be due either to IUD use in the post-MR period or to complications of the MR procedure itself. This question, however, could be answered by incorporating a control group of MR patients who are non–IUD users. Unfortunately our study did not contain such a group.

The higher infection rate among patients with induced abortion is due understandably to the unhygienic and unsanitary abortion procedure. Therefore, by substituting the modern abortion induction techniques for unhygienic indigenous abortion procedures, it should be possible to substantially reduce the rate of pelvic infection.

Etiologic factors in pelvic inflammatory disease in Sudanese women

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Pelvic inflammatory disease constitutes a major gynecologic problem in the Sudan as in most African countries. This article reviews studies of three important causes of PID involving Sudanese women. Women who attended the gynecologic clinic of Khartoum Hospital with a complaint of vaginal discharge were tested for gonococcal infection to evaluate this potential etiology; only 1.4% of these women had gonorrhea. Since septic abortions also result in PID, we studied 2,447 women admitted to Khartoum hospitals in 1976; only 0.4% of these women had had septic abortions. Female circumcision, which is widely practiced in the Sudan, is the subject of an ongoing epidemiologic study. The clinical impression is that this harmful tradition is an important cause of PID among Sudanese women. (AM. J. OBSTET. GYNECOL. 138:877, 1980.)

PELVIC INFLAMMATORY DISEASE is one of the persistent clinical problems that faces the Sudanese gynecologist in his ordinary practice. It is estimated that about one third of the women reporting to gynecology clinics in the major hospitals suffer from PID or its sequelae, mainly infertility. This, of course, imposes an even greater burden on the already scarce and deficient health facilities of the country. It is incumbent on the professional cadres delivering medical care to the community to evaluate the problem, determine its magnitude, and suggest ways for its prevention.

This article discusses PID in Sudanese women with regard to three etiologic factors: prevalence of gonococcal infection, abortions, and female circumcision.

Sexually transmitted diseases

It is recognized that sexually transmitted diseases (STDs) are often the cause of PID. To discover the importance of STD, women reporting with vaginal discharge to Khartoum Teaching Hospital were referred to a special clinic where proper examinations and bacteriologic tests were performed.

A study was made of 147 patients who were between 15 and 45 years old, with 75% between the ages of 20 and 30 years.[1] Ninety percent of these women were married and 60% had children. Only six patients were taking contraceptive pills and four were wearing an intrauterine contraceptive device. All but one of the patients were circumcised; the pharaonic type was the most common by far.

Table I. Isolation of genital organisms from Sudanese women with vaginal discharge at Khartoum Teaching Hospital, 1976

Organism	*Patients (N = 147)* No.	%
Candida species	48	32.6
Trichomonas vaginalis	13	8.8
Candida and *Trichomonas vaginalis*	5	3.4
Neisseria gonorrhoeae	2	1.4
Gram-positive bacilli	45	30.6
No organism isolated	34	23.0

Neisseria gonorrhoeae was isolated from only two women (1.4%) with vaginal discharge in our study and trichomoniasis was discovered in only 18 (12%) (Table I). This is in sharp contrast to the epidemic rates of gonorrhea reported throughout Europe and North America.[2] We attribute this to the fact that the majority of these patients were married and had stable sexual relationships. However, more elaborate studies with larger patient samples and appropriate laboratory screening outside hospital clinics will be needed to draw concrete conclusions for the Sudan.

Hospital abortion

Septic abortions are considered among the most serious etiologic factors involved in PID.

In Khartoum in 1976, 2,447 patients treated for in-

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Table II. Distribution of 2,447 women at admission to Khartoum Hospitals by abortion type, 1976

Type of abortion	*Omdurman (N = 707)* No.	%	*Khartoum General (N = 931)* No.	%	*Khartoum North (N = 809)* No.	%	*Three-Town City (N = 2,447)* No.	%
Spontaneous, incomplete	683	96.6	921	98.9	791	97.8	2,395	97.9
Induced	20	2.8	7	0.8	6	0.7	33	1.3
Spontaneous, incomplete with systemic condition	4	0.6	3	0.3	12	1.5	19	0.8

complete abortion were followed to evaluate the clinical management and complications of this condition.[3] Most of the 2,447 abortion cases were the spontaneous, incomplete type and very few were induced abortions, possibly due to the existing restrictive abortion laws in this country (Table II).

Septic abortion was diagnosed when patients had a temperature of ≥100.4° F for 2 days or a single temperature reading of 102° F. Some cases with temperature criteria were not diagnosed as septic abortion when a common cause of fever (e.g., malaria) was recognized. However crude these criteria, only 10 cases (0.4%) of septic abortion were identified. When compared with similar studies in other countries, this is an exceptionally low incidence of septic abortion.[4-6] In a comparison between the patients in this study and patients in a similar study in Jakarta, Indonesia, it was found that 17.1% of the cases seen at the Jakarta hospital were septic. This difference might be due to the incidence of illegally induced abortion in the two areas or a difference in the patients' access to hospital care. The widespread use of antibiotics and their availability without prescription in the Sudan might mask cases of septic abortion among patients with illegally induced abortions, prior to their admission to the hospital. In fact, the usual practice of persons performing illegal abortions seems to be to administer an antibiotic cover to reduce the possibility of infection.

Further studies must evaluate the incidence of septic abortions using more refined criteria. Also, a long-term follow-up of patients with septic abortions is needed to determine the incidence of PID and its sequelae.

Female circumcision

Female circumcision is one of the harmful traditional practices still practiced widely in the Sudan. It is performed on girls 7 to 9 years of age in three types of procedures:

1. *Sunna circumcision* involves removal of the prepuce and tip of the clitoris, an operation that requires some skill.
2. *Excision or clitoridectomy* (most frequently performed) includes excision of the clitoris, labia minora, and sometimes all external genitals.
3. *Infibulation or pharaonic circumcision* (excision and infibulation, formerly the most common type in the Sudan) involves removal of the entire clitoris, the entire labia minora, and the adjacent medial parts of the labia majora in their anterior two thirds at least. The two sides of the vulva then are brought together by silk or catgut sutures, obliterating the vaginal introitus except for a small opening posteriorly to allow the passage of urine and later of menstrual flow.

This practice has a number of complications which can be summarized:

1. *Immediate:* shock due to pain and/or hemorrhage; infection of the wounds; urinary retention; damage to urethra or anus; and hematocolpos
2. *Late:* keloid formation and implantation dermoid cysts, including abscesses; chronic pelvic infection; calculus formation; dyspareunia; infertility; urinary tract infection; and difficulty of micturition
3. *Obstetric:* perineal laceration; consequences of anterior episiotomy including blood loss, injury to the bladder, urethra, or rectum, and puerperal sepsis; delay in labor and its consequences; vesicovaginal fistula, rectovaginal fistula; fetal loss; and fetal brain damage.

PID seems to be one of the complications of female circumcision. Shandall[7] reviewed 436 cases of nontuberculous chronic pelvic infection. He noticed that the incidence of PID in patients with pharaonic circumcision was more than three times that in the sunna circumcision group. He suggested that infibulation causes PID in three ways: (1) infection at the time of circumcision resulting from the septic conditions under which this operation is performed, (2) interference with drainage of urine and secretions from the vagina, which might result in endometritis and adnexal infection, and (3) infection of the split and resutured circumcision wound after labor. (The scar is split toward the end of the second stage of labor and the circumcision wound is resutured after labor.)

Although there is controversy about the relation of circumcision to PID, it is now generally recognized to be an important etiologic factor for PID. When considering the medical problems of women following this practice, PID should be included. I feel circumcision is the cause of a great deal of suffering among Sudanese women. A more rigorous study is now in progress in the Sudan to evaluate the complications of this practice more precisely. There is also a plan to review the attitude of the society in an effort to formulate ways of abolishing this custom.

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EPIDEMIOLOGY

Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries

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Pelvic inflammatory disease is used herein synonymously with acute salpingitis. In modern industrialized countries, the annual incidence of PID in women 15 to 39 years of age seems to be 10 to 13 per 1,000 women, with a peak incidence of about 20 per 1,000 women in the age group 20 to 24 years. Since 1960 an increase in incidence by a factor of 1.6 to 1.9 has been observed in the age group 20 to 29 years. The incidence of PID is correlated strongly with the prevalence of sexually transmitted diseases, although a fraction of the infections might be of endogenous origin. Use of intrauterine contraceptive devices and operations for legal abortions contribute to the increase in incidence. The prevalence of women in the post-PID state has increased by a factor of about 1.5 since 1960. Women in the post-PID state have a tenfold increased risk for ectopic pregnancy and 25% of the increase in ectopic pregnancy can be accounted for by the increase in post-PID women. Infertility after PID ranges between 5.8% and 60% depending on severity of infection, number of infections, and age of the woman. The fraction of women rendered infertile because of PID has increased by a factor of about 1.6 since 1960. (AM. J. OBSTET. GYNECOL. 138:880, 1980.)

NONTUBERCULOUS infections of the pelvic organs are common in young women. There is general agreement that the vast majority of such infections are caused by ascending spread of an infection in the lower genital tract.[1, 2] In some cases of pelvic infection, the woman's future reproductive ability will be impaired. In this respect, the infections of the fallopian tubes (salpingitis) are the most important. Postinfection tubal damage is an etiologic factor in 30% to 40% of cases of female infertility[3, 4] and in 40% to 50% of cases of ectopic pregnancy.[5, 6] There is a consensus of opinion that the incidence of tubal infections currently is increasing, and, consequently, the prevalence of women with postinfection tubal damage. The increased number of tubal infections has been "explained" by referring to the current epidemic of sexually transmitted diseases (STD) and the fact that a large proportion of salpingitis cases are caused by microbial agents that are transmitted during sexual intercourse.

It has been questioned whether the postulated epidemic of pelvic infections has a causal relationship to the increases observed during the last decade in ectopic pregnancy[7, 8] and female infertility.[9] For many reasons, analyses are difficult to perform on the prevalence and trends of the pelvic infections and their sequelae and, consequently, on their impact on modern society.

Terminology and definitions

For practical purposes, mainly because an exact diagnosis is not always possible, the pelvic infections—including salpingitis—often are grouped together

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Table I. Correlation between clinical criteria for PID and the final laparoscopic diagnosis

Criteria for suspicion of tubal infection	*Laparoscopic diagnosis/100 cases*		*% All salpingitis cases*
	Tubal infection	*Normal tubes*	
Minimum: all three of (1) signs of genital infection, (2) low abdominal pain, and (3) pelvic tenderness	61	39	16.1
In addition: one or more of (1) erythrocyte sedimentation rate >15 mm/hr, (2) rectal temperature >38.0° C, and (3) palpable adnexal mass			
Minimum +1	68	32	28.3
Minimum +2	90	10	38.7
Minimum +3	96	4	16.9

using the term "pelvic inflammatory disease." Unfortunately, this is a term that lacks a precise definition. In official reports, PID usually includes one or more of the World Health Organization (WHO) International Classification of Diseases (ICD) groups 612, 613, 614, and 616 (acute, chronic, and unqualified salpingitis and diseases of the parametrium and pelvic peritoneum, respectively). This definition of PID includes some noninfectious disorders such as pelvic adhesions and so-called chronic salpingitis, whereas in many reports gonorrheal infections (ICD098) as well as salpingitis after abortions (ICD645) are excluded.

In the clinics, PID usually is used to define an acute febrile illness in a woman with pelvic pain and signs of a genital infection. Using this definition, many cases of mild salpingitis are excluded. In our experience, many cases of salpingitis are afebrile and have a very benign clinical course.

This uncertainty of definitions highlights the most difficult problem pertaining to epidemiologic studies on acute salpingitis, viz., our lack of simple, safe, and specific diagnostic methods. Consequently, the criteria used to define the condition vary.

In the following discussion, the term PID will be used synonymously with acute salpingitis despite the fact that the different reports referred to might include other diseases as well as exclude some salpingitis cases.

Pathophysiologic and etiologic aspects

It is customary to subdivide acute salpingitis into "gonococcal" salpingitis and "nongonococcal" salpingitis, depending on whether or not gonococci can be isolated from the lower genital tract. Reports from the last decades have clearly demonstrated that acute salpingitis has a multifactorial microbial etiology,[10, 11] and that sexually transmittable agents other than *Neisseria gonorrhoeae*, such as *Chlamydia trachomatis*[12, 13] and *Mycoplasma hominis*,[14, 15] might be isolated from the fallopian tubes in cases of PID. Furthermore, anaerobic bacteria have been isolated from cul-de-sac aspirates in some PID cases.[16, 17] Many of these anaerobic species belong to the endogenous vaginal and perianal flora.[18]

The vast majority of salpingitis cases are caused by ascending spread of an infection from the lower genital tract.[1, 2] The mechanisms of the ascent of the cervical infection to the tubes are, however, not fully understood. It has been suggested[19] that a "cervical resistance factor" protects the individual against ascent of a cervical infection. This factor should disappear during ovulation and menstrual bleeding. This hypothesis fits with the observation that salpingitis—especially in cases with cervical gonorrhea—often appears at menstruation[11, 20] and that the use of oral contraceptives—which inhibit ovulation—seems to protect women from ascent of a cervical infection.[11, 21] Because salpingitis is not seen in women who are not sexually active,[22, 23] it has been suggested[24] that uterine coital contractions facilitate the spread of cervical contents into the uterine cavity and further to the tubes.

In some cases, iatrogenic procedures such as legal abortions and insertion of intrauterine contraceptive devices (IUDs) can cause an unrecognized infection in the cervix to spread to the uterine tubes.[25]

A small proportion of the tubal infections are caused by direct spread from a primary focus outside the genital tract, usually the appendix.[25]

For a better understanding of the epidemiology of acute PID, it seems logical, therefore, to subdivide the salpingitis cases into:

I. *Primary salpingitis*—an infection which ascends from the lower genital tract. This type can be caused by:
    A. *Exogenous* agents or factors
        1. *Venereal PID*, i.e., infections caused by sexually transmitted microorganisms
        2. *Iatrogenic PID*, i.e., spread of a cervical infection to the uterine tubes by diagnostic or therapeutic procedures or by IUD insertions
    B. *Endogenous* agents, i.e., ascending spread of the endogenous vaginal or perianal flora
II. *Secondary salpingitis*—an infection of the fallopian tubes caused by direct spread from nearby pelvic organs, most often the appendix

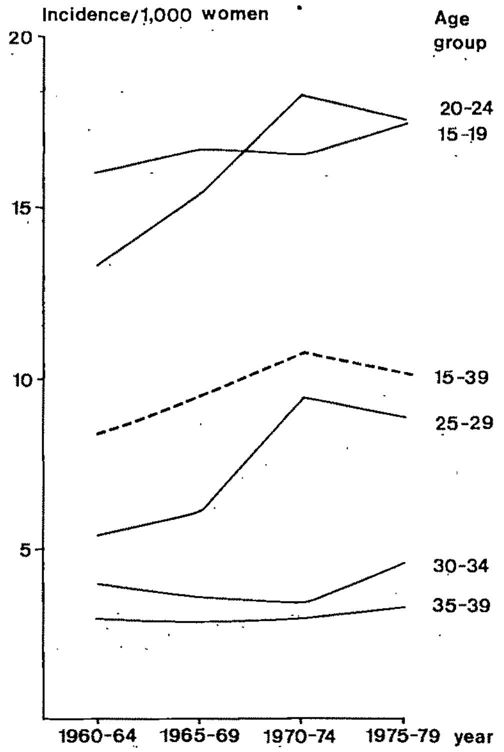

Fig. 1. Mean total annual incidence of acute salpingitis per 1,000 women in the city of Lund during the periods 1960-64, 1965-69, 1970-74, and 1975-79.

From an epidemiologic point of view, it seems logical to assume that the primary endogenous infections as well as the secondary infections should show a relatively constant prevalence, whereas the "venereal" and "iatrogenic" infections should vary with the epidemiology of the STDs in general as well as with the frequency of procedures such as IUD insertions and (legal) abortions.

Therefore, analyses of the epidemiology of PID and its sequelae as well as its impact on the modern society should concern primarily the relationships between the STDs, "iatrogenic" factors, and PID.

Incidence and trends of PID

Factors influencing the reported numbers of PID. As mentioned, our lack of simple and specific diagnostic methods is the main obstacle for a true knowledge of the prevalence and trends of salpingitis. Routine use of laparoscopy on liberal clinical grounds has revealed that the commonly used clinical criteria for the diagnosis of tubal infections have a low level of accuracy (Table I).[26] Many salpingitis patients have atypical symptoms and signs of disease; indeed, some patients with tubal infection have no symptoms at all.[11, 27] A consequence of the mildness of symptoms of many tubal infections is that patients consult doctors in places other than hospital emergency rooms, often are treated on an outpatient basis, and are never reported as having salpingitis. As a result, underreporting is common. Furthermore, because many of the clinically benign salpingitis cases are caused by *Chlamydia trachomatis*,[28] our analyses of the relationship between STDs and PID are distorted.

Educational levels of both patients and doctors and economic factors also influence the patient to consult a doctor and the doctor to report the case. Differences in the organizations and capacities of the health care systems as well as the reporting systems make comparisons between different countries and regions difficult or impossible.

Because of these difficulties, reported prevalence data for PID are estimates extrapolated from small samples or calculations based on reported figures on uncomplicated gonorrhea, gonococcal PID, and hospitalized patients with PID.

Ideally, determinations of the incidence of salpingitis should be based on case reports confirmed by laparoscopy utilized on liberal clinical criteria for all women presenting with symptoms suggestive of a tubal infection. To our knowledge, this approach has been the rule only in our hospital catchment region (discussion to follow).

Reported figures on the incidence of PID from industrialized countries. Between the two world wars, the number of women hospitalized with PID (suppurative adnexitis) decreased in most European countries.[19, 29] In those days, 30% to 80% of the PID cases were associated with gonorrhea.[20] Much of the decrease in the immediate pre–World War II era was ascribed to the advent of chemotherapy.

After World War II, a steady increase in the number of women hospitalized with PID was observed in most European countries. The major part of this increase was seen during the last two decades. Much of the increase can be attributed to elimination of economic factors, i.e., to better social security systems with, for instance, free hospital care.

Attention also has been drawn to the epidemic of STD, especially gonorrhea, during this period. In the Scandinavian countries and England and Wales, the reported number of cases of gonorrhea has trebled since the mid-1950s. From 1973 to 1976, the mean annual rates of reported cases of gonorrhea per 100,000 population were 300 to 350 in the Scandinavian countries; 120 to 230 in Germany, England and Wales, and Northern Ireland; 34 in France; and between eight and 11 in the Catholic countries (Italy, Spain, Portugal, Ireland, Belgium).[30]

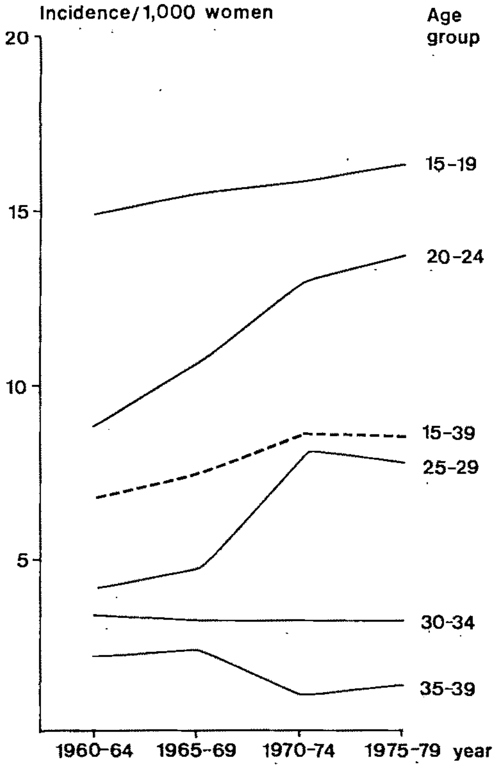

Fig. 2. Mean annual incidence of first episodes of salpingitis per 1,000 women in the city of Lund during the periods 1960-64, 1965-69, 1970-74, and 1975-79.

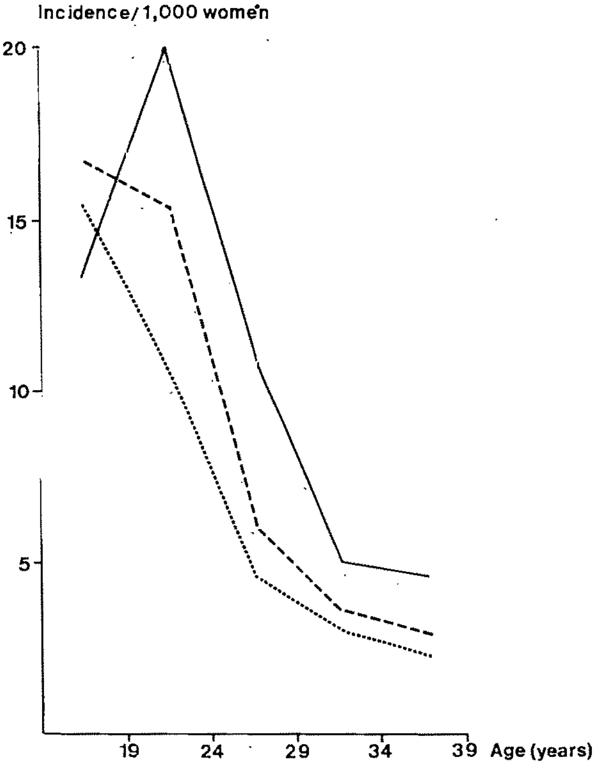

Fig. 3. Age-specific rates of PID per 1,000 women in Atlanta, Georgia, and Lund in 1965. *Solid line,* Wright and Laemmle in Atlanta, first episodes (AM. J. OBSTET. GYNECOL. 101:979, 1968); *broken line,* Weström in Lund, total incidence; *dotted line,* Weström in Lund, first episodes.

However, the incidence of not only gonococcal-associated PID but also nongonococcal PID has increased,[20] and a trend toward a lower proportion of gonococcal PID has been reported.[20, 23]

From 1950 to 1965, a doubling of the number of hospital-treated PID patients was observed in the Scandinavian countries.[20, 31] In England and Wales, hospital discharges due to PID (ICD groups 612 to 614) per 100,000 women increased from 1964 to 1972 by a factor of 1.3.[32]

In Europe, as in the United States, PID is not a reportable condition, and there are, to our knowledge, no European estimates of the total prevalence of salpingitis, apart from those made by us. At the Department of Obstetrics and Gynecology in Lund, Sweden, we have studied various aspects of PID since 1960. Earlier, we published a demographic description of the city and its population changes since 1960.[23] In brief, the city of Lund is dominated by public institutions and a university, but has no major industry. The registered population on December 31, 1979, was 78,678 persons. The number of women between 15 and 39 years of age increased from 12,077 in 1960 to 16,411 in 1979. Because of the university, the age group 20 to 29 years dominates the female population. Our clinic is the only gynecologic clinic in the city, and all women with gynecologic complaints, including all cases of assumed salpingitis, are referred to the clinic for examination. The patient data analyzed here include all women between 15 and 39 years of age, registered in the city, in whom acute salpingitis was diagnosed from 1960 through 1979. The diagnosis of salpingitis was verified routinely by laparoscopy. Laparoscopy was performed within 24 hours of admission to the hospital and before any treatment was instituted. The laparoscopic technique, the criteria for performing a laparoscopy, the criteria of a laparoscopic diagnosis of salpingitis, and the grading of the inflammatory reactions of the fallopian tubes as seen at laparoscopy have been described in detail elsewhere.[11, 23, 33]

During the period of study 2,776 episodes of acute salpingitis were diagnosed in 2,193 women in Lund.

The total annual incidence of PID per 1,000 women 15 to 39 years of age increased from 8.3 in 1960-64 to 10.7 in 1970-74. Thereafter it decreased to 10.0 in 1975-79 (Fig. 1). As can be seen from the figure, the incidence was highest in the age groups 15 to 19 and 20 to 24 years. The increase was most marked in the age groups 20 to 24 and 25 to 29 years (increases by factors of 1.4 and 1.8, respectively). After 1974, a slight decrease was observed in women between 20 and 29 years of age, whereas an increase was noted in the teenage group and in women 30 to 34 years of age (Fig. 1).

Throughout the period of study, the annual incidence of first episodes of PID (Fig. 2) was highest in the teenage group; thereafter it decreased with increasing age of the women. Like the total incidence, the incidence of first episodes of PID increased most in the women 20 to 24 and 25 to 29 years of age (increases by factors of 1.6 and 1.9, respectively).

The incidence of first episodes of PID also denotes the rate of women who have ever had salpingitis, i.e., women in the post-PID state. For an epidemiologic analysis of the sequelae of PID, the magnitude of that fraction of women is most important. For example, from Fig. 2 it can be calculated that in 1969, 127 out of 1,000 25-year-old women were in the post-PID state. In the age group 20 to 29 years, the prevalence of women in the post-PID state increased from 9.8% in 1960-64 to 15.9% in 1975-79.[23]

Wright and Laemmle[34] estimated the incidence of PID in an indigent population in Atlanta, Georgia, in 1965. They found a peak incidence of 20.0 per 1,000 women in the age group 20 to 24 years. A comparison of the age-specific annual incidences of PID between Lund and Atlanta in 1965 is shown in Fig. 3. There is a striking similarity between the two estimates.

Using data from the National Disease and Therapeutic Index (NDTI) study, as well as the Commission on Professional and Hospital Activities, Eschenbach and colleagues,[21] using two methods, estimated the total number of PID cases in the United States from 1970 through 1974 to be between 505,800 and 555,814. These figures correspond to an annual incidence of 13.6 cases of PID per 1,000 women between the ages of 14 and 34 years.[21] The corresponding figure for Lund during the same time period and in the same age group was 11.9 (Fig. 1).[23]

Using our figure of 10.7 PID cases per 1,000 women per year in the age group 15 to 39 years and applying that figure to the total female population of reproductive age in the United States (42 million in 1976), we arrive at a total number of 450,000 PID cases annually in this country. Based on the reported number of cases of female gonorrhea in the United States, Urquhart[8] arrived at an estimated number of 800,000 PID cases annually (1979).

The NDTI has estimated the rate of visits to office-based private physicians for PID (ICD groups 612, 613, 614, and 616) to be about 35 visits per 1,000 female population 10 to 50 years of age in the early 1960s, increasing to 45 in the early 1970s.[35] In 1973 to 1976, data from the National Ambulatory Medical Care Survey produced a mean of 1,900,000 such visits annually. These figures might, however, overestimate the true number of women afflicted with acute salpingitis because of the ICD diagnoses chosen and the fact that a disease period might include more than one visit.

If we, as a conservative estimate, assume half a million new cases of acute salpingitis annually in the United States, then a corresponding number of women will add to the prevalence of women in the post-PID state. If that rate continues, it is destined within a short period to bring the proportion of women of reproductive age who are in the post-PID state to an aggregate level close to 15%, i.e., the same as in Lund (see previous discussion).

We know that at least one out of four women in the post-PID state will suffer from one or more of the sequelae of chronic abdominal pain, infertility, and ectopic pregnancy.[23] If we accept a figure of 10% to 15% of women of reproductive age as being in the post-PID state, then it is obvious that PID has a great impact on the disease pattern, human suffering, and economy in modern society.

Factors influencing the epidemiology of PID

STD and PID. Numerous reports have called attention to the current worldwide epidemic of STD.[8, 36-40] In women, STDs cause infections primarily in the lower genital tract. Consequently, the incidence of STD-related cervicitis and vaginitis currently is increasing. Salpingitis is caused, in the vast majority of cases, by ascending spread of an infection from the lower genital tract.[1, 2] Although direct proof of an STD-related increase in PID is lacking, there is indirect evidence that a large proportion—in some areas and age groups the majority—of PID cases are caused by agents that are transmittable via sexual intercourse. The following are some of the main facts regarding STD and acute salpingitis:

1. A large proportion of the microorganisms which have been isolated from the uterine tubes in cases of salpingitis are sexually transmitted.[10-14, 41-44]
2. A causal relationship between STD and acute salpingitis was demonstrated in most PID cases in the age group 15 to 24 years in our area.[24]
3. Some 10% to 19% of women with cervical gonorrhea develop acute salpingitis.[20, 23, 45]
4. Cervical gonorrhea has been diagnosed in half of all PID cases in some areas in the United States.[39]
5. The age distribution of women with acute salpingitis corresponds to that of women who have STD confined to the lower genital tract.[41, 46, 12]

6. The prevalence of genital infections and their complications is related to the sexual activity of the individuals and to the numbers of sexual partners.[47–49]

7. Acute salpingitis is a rarity in women who are not sexually active.[22, 23]

Among STDs which can give rise to salpingitis are *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Mycoplasma hominis.*

Since 1960, the incidence of gonorrhea in the United States has increased by a factor of 2.7.[50] During 1971-72, the number of hospitalized patients with PID increased in proportion to the increase in female gonorrhea.[37] In Sweden, the prevalence of uncomplicated gonorrhea increased through the 1960s to an all-time high in 1970, followed by a decrease.[23] The decrease was accompanied by a concomitant decrease in the annual incidence of gonococcal PID.

However, uncomplicated gonorrhea in the female patient cannot be taken as a marker of the epidemic of PID in general. As is evident from Fig. 4, the incidence of gonococcal PID in Lund has decreased, whereas the total incidence of PID has increased. Furthermore, the rate of salpingitis in women with cervical gonorrhea has decreased in our region from 20.3% in 1960-64 to 9.7% in 1975-79. Similar observations also have been made by others.[51]

Evidence that genital chlamydial infections are increasing can be found in the observed increase in nongonococcal urethritis (NGU) in men. In 40% of the NGU cases, the cause is *C. trachomatis.*[52] NGU has shown a three- to sixfold increase in different parts of the world during the last decade.[37, 39, 53, 54, 56] Chlamydial infections of the cervix are reported with increasing frequency.[55–57]

In 1977, we isolated *C. trachomatis* from the uterine tubes in six out of 20 cases of acute salpingitis.[12] Further studies[42–44, 58] have established *C. trachomatis* as an important etiologic agent in PID. Over the last few years, we have collected evidence that more than half of the PID cases in women less than 25 years of age in our city were caused *C. trachomatis.*[53]

During 1967-71, we studied the role of *M. hominis* in acute salpingitis.[14, 46] The results suggested that during that period, *M. hominis* was associated with 10% to 15% of the tubal infections in our region.[42]

These three microorganisms are transmitted during sexual intercourse. In our area these microorganisms have accounted for more than 75% of all PID cases in women less than 25 years of age during the last few years. In view of these interrelations between STD and PID, the current epidemics of genital chlamydial infections and gonorrhea seem to predict a further increase in the incidence of PID and, consequently, an increasing prevalence of women in the post-PID state.

From the aforementioned, it seems obvious that the STDs have an important—probably the most important—impact on the epidemiology of salpingitis. Therefore, any attempt to decrease the incidence of PID must start with attempts to decrease the incidence of STD in general. Furthermore, an etiologic diagnosis and epidemiologic follow-up, including partner treatment, are as important in PID as they are in STD in general.

Age, sexual activity, and PID. The age distribution of women with PID is the same as that in women with uncomplicated STD.[12, 41, 46] In our series, 68.9% of the total number of women with tubal infections were younger than 25 years. Of the total number of women, 33.1% had their first infection before the age of 20 years,[23] i.e., before starting their reproductive period in life. Among all infected women 74.4% were nulliparous. In view of this, it is obvious that the majority of cases of post-PID infertility will be primary infertility, i.e., most women rendered infertile because of salpingitis will never have children of their own.

In women who are not sexually active, e.g., nuns, only tuberculous salpingitis and PID secondary to appendicitis have been observed.[22] In 1964, Viberg[59] emphasized the nonvirginity of his patients with salpingitis. In our patients, all PID patients admitted coital experience and the clinical examinations revealed none of them to be a virgin.[23]

In the female population, most women over 20 years of age are sexually active, whereas a large proportion of the teenagers are not. Therefore, the incidence figures presented (Figs. 1 and 2) are not comparable between the age groups in terms of risk estimations for PID. In a separate analysis of the salpingitis patients 15 to 24 years of age during 1970-74, and based on determinations of the proportion of women of different ages with coital experience,[60] we found a highly significant decrease in the incidence of PID with increasing age in women who were regarded as sexually active.[23] Thus, the risk of acquiring salpingitis in sexually active 15-year-old girls was calculated to be 1:8, and in girls aged 16 years, 1:10. The incidence then rapidly declined and was 1:80 in women aged 24 years.[23]

A correlation between early coital debut and promiscuous sexual relations might explain the very high salpingitis incidence in sexually active teenage girls. In the study mentioned,[60] it was found that among 15-year-old girls with coital experience, 50% had had four or more partners during that year while only 20% of women 18 years of age were as promiscuous.

In a survey of data presented on the sexual behavior

Table II. PID cases and rates per 100 women 20 to 29 years of age per year, by woman-years of method of contraception (Lund, 1970-74)

		Contraceptive users			*Non-contraceptive users*		
	Total	*IUD users*	*OC users*	*Barrier users*	*Sexually active*	*Sexually inactive*	*Pregnant*
Woman-years	41,930	2,935	16,311	7,044	5,032	5,031	5,577
PID cases (no.)	571	153	148	98	172	0	0
PID/100 woman-years	1.36	5.21	0.91	1.39	3.42	0	0

of American college women,[49] it was found that until 1965 a relatively constant 20% to 22% of such women had engaged in premarital sexual intercourse by the age of 20 years. After 1965 a rapid and significant increase to 40% to 44% was reported. It is obvious that such a change in sexual behavior in young women must have implications for the prevalence of STD in general and, hence, also for PID.

"Iatrogenic" PID. Opening of the cervical canal or the introduction of foreign material into the uterine cavity facilitates ascending spread of infection from the cervix.[61] Such procedures include cervical dilatation, abortion, exeresis, curettage, tubal insufflation, hysterosalpingography, and IUD insertion. The rates of infectious complications for such procedures depend on precautions and technique, but usually are low.

During the last two decades, the numbers of legal abortions and IUD insertions have reached such proportions that their immediate infectious complications might influence the epidemiology of PID.

In our clinic, first-trimester legal abortions are performed using vacuum aspiration on an outpatient basis. We have observed a rate of about 10% immediate (within 5 days) febrile reactions after such operations. In cases of immediate postoperative febrile reactions, laparoscopy in all but one of 145 cases[62] revealed normal intrapelvic organs. True salpingitis usually appears more than 5 days after the operation, and in general has a more insidious onset. In our experience with legal abortions, 0.5% are complicated with acute salpingitis within 3 weeks of the operation.[62] With nearly one million legal abortions annually (1976) in the United States,[63] this corresponds to 5,000 cases of salpingitis each year.

As to the IUDs, not only the procedure of insertion but also the use of the devices imply a risk of ascending spread of a cervical infection (discussion to follow).

In our series of PID cases, "iatrogenic" factors preceded the onset of PID within less than 4 weeks in about 12% of the cases.[11, 21] Between 1960 and 1967, exeresis after spontaneous abortions accounted for six out of 10 such cases, whereas later, IUD insertions and legal abortions accounted for five and three out of 10, respectively.[11]

Endogenous PID. In our series of PID cases, the rate of proven STD-associated PID was lower in the age groups 30 years and older than in women under 25 years of age.[15, 44, 46] This suggests that, from an etiologic point of view, STDs are less important for PID in the somewhat older women. On the other hand, infections caused by anaerobic bacteria seem more prevalent in the older PID patients[23] and also in women who have had two or more episodes of PID.[64] The rationale for this might be that a post-PID uterine tube is more vulnerable to infections by anaerobes.[64] Anatomic changes induced by pregnancy and delivery contribute to an easier access to the vagina for bowel flora. This might make possible an increased occurrence of a type of endogenous nonvenereal PID in women of comparatively greater age.

Secondary PID. Pelvic infections originating in structures outside the genital tract were rare in our series (less than 1%).[25] Such infections should, in any series, have a stable and low incidence.

Contraceptive usage and PID. Several studies have revealed that IUDs were used significantly more often by women with PID than by control women.[21, 65–68] Statistical analyses have indicated that the risk of acquiring PID in nulligravid IUD-using women is seven- to ninefold that of nulligravid women who do not use IUDs.[21, 67, 68] Age, race, socioeconomic status, and sexual activity are also of importance for differences in the risks of PID in IUD users vs. nonusers.[68] In Seattle, Washington, 22.3% of all cases of PID in 1972 were calculated to be directly caused by IUD use.[21] It also has been observed that use of oral contraceptives (OCs) was reported significantly less often by women afflicted with acute salpingitis than by control women.[21, 65–67]

In a study from Linköping, Sweden,[69] comprised solely of women with cervical gonorrhea, it was found that IUD-using women developed salpingitis significantly more often than did those using OCs. The results from this study argue against the suggestion that women choosing IUDs primarily originate from a group at higher risk of acquiring STD and/or PID.

Table II presents data on the use of contraceptives among all 20- to 29-year-old women in Lund, 1970-74, compared with data on the use of contraceptives in patients with PID during the same period and in the same age group. It appears that the use of an IUD increased the risk of acute salpingitis, whereas the use of OCs or barrier methods seemed to protect the women from PID, when a comparison was drawn with the PID risk in sexually active women who used no contraceptive method. When the data in Table II were divided as to nulligravid and gravid women, the PID rates for the OC users, for users of barrier methods, as well as for the non-contraceptive users were the same as in the total sample. The risk of acquiring PID in nulligravid IUD users, however, was 11.8 per 100 woman-years of use and 3.4 (i.e., the same as in non-contraceptive users) for gravid IUD users. It should be emphasized that sexually active women who use no contraceptive method constitute the correct reference group to be used when determining the effects of different contraceptive methods on the epidemiology of genital infections. It also must be admitted that IUDs were used rather uncritically in young nulligravid women in our area at that time.

Thus widespread use of IUDs might have an impact on the epidemiology of PID. Based on the figures of IUD usage among PID patients in Seattle and IUD usage in that region, calculated numbers of PID cases in the United States, and percent IUD usage in the United States, Eschenbach and associates[21] calculated that in 1972, 110,000 PID cases per year were accounted for by IUD usage. Had those women used OCs instead of IUDs, about 80,000 of them would never had acquired PID (Table II).

The protective effect of OC usage against the ascent of a cervical infection which is suggested by the epidemiologic findings, might be explained by the maintenance of the cervical mucus barrier throughout the menstrual cycle, the short, scant menstrual bleedings from an inactive endometrium, and decreased muscular activity of the myometrium.[23]

Sequelae to PID

Mortality. The prognosis of acute salpingitis quo ad vitam is good. In 1930, Holtz[70] reported a mortality rate of 1% in a series of 1,300 cases of nontuberculous salpingitis. Since the advent of antibiotic therapy, deaths have been reported only occasionally. Today, death from salpingitis generally is due to rupture of tubo-ovarian abscesses with a generalized peritonitis.[71, 72] In such instances, the mortality rate is still as high as 8.6%.[72] In Sweden, one death from acute salpingitis has been reported since 1960. In our studies, the mortality rate was nil.

Chronic abdominal pain. Women in the post-PID state who complain of chronic abdominal pain are well known to every gynecologist. In such patients, laparoscopy usually reveals dense pelvic adhesions locking the fallopian tubes and the ovaries. The cause of the pain is not quite understood, but one reason might be increased intraovarian pressure induced by cycle-related volume changes, which are made difficult by adhesions surrounding the ovaries. The comparatively good results with continuous progesterone or danazole treatment strengthen this hypothesis.[62]

Chronic abdominal pain, probably as a late effect of a previous bout of salpingitis, was reported by 17% of the PID patients studied by Falk.[2] Such pain, lasting for longer than 6 months and causing the woman to seek medical advice, was reported by 18.1% of the women in a follow-up study of PID in our series.[33] The corresponding figure was 5% in a series of control women who had never had PID.[33] Of the women who suffered from chronic pelvic pain after PID, the majority (63%) also were involuntarily infertile and an equal proportion (68%) also had dyspareunia. With half a million new cases of PID annually, these figures might mean that each year 90,000 post-PID women with chronic abdominal pain are added to the already existing pool of such women in the United States.

Infertility. Acute salpingitis has long been known as one of the major causes of female infertility.[3, 4] Before the advent of antibiotic therapy, Holtz[70] reported a pregnancy rate of 25.4% in married women after acute salpingitis. In 1958, Hedberg and Speyz[3] reviewed 216 cases of salpingitis. They found a pregnancy rate of 60% after the exclusion of unmarried and voluntarily childless women. In 1965, Falk[2] noted a pregnancy rate of 81% after corresponding exclusions.

The extent to which the tubal infections lead to infertility has been studied prospectively by us.[33, 73, 74] In the first study[33] 415 women proven laparoscopically to have had salpingitis were monitored for a period extending to 1974. This study included *all* pregnancies diagnosed during the period of observation in the PID group as well as a control group of 100 women who had never had PID. In the second study,[73] 751 women treated for laparoscopically verified PID between 1968 and 1974 were followed to their *first* pregnancy or, if not pregnant, up to 1979. This study included comparisons between the different antibiotic regimens

Table III. Reproductive events after one or more tubal infections in 1,000 women 15 to 34 years of age (Lund, 1960-74), followed to first pregnancy or, if not pregnant, for 8 years

	No./1,000 women
Pregnant	
Intrauterine	624
Ectopic	41
Not pregnant	
Voluntarily	171
Involuntarily	
Tubal occlusion	126
Other causes	38

Table IV. Percent of infertility because of tubal occlusion after one, two, and three or more episodes of salpingitis in women exposed to a chance of pregnancy

No. of infections	*% Infertility postsalpingitis in age group*		
	15-24 yr	*25-34 yr*	*Total*
1	9.4	19.2	11.4
2	20.9	31.0	23.1
3+	51.6	60.0	54.3

Table V. Percent infertility because of tubal occlusion after mild, moderately severe, and severe infections in women with one episode of salpingitis and exposure to a chance of pregnancy

Inflammatory changes	*% Infertility postsalpingitis in age group*		
	15-24 yr	*25-34 yr*	*Total*
Mild	5.8	7.8	6.1
Moderately severe	10.8	22.0	13.4
Severe	27.3	40.0	30.0

Table VI. Calculated rates of post-PID infertility in cohorts of 1,000 women born 1940, 1945, 1950, and 1955

Cohort born in	*Cumulative rate of post-PID infertility at age*				
	19 yr	*24 yr*	*29 yr*	*34 yr*	*39 yr*
1940	8.55	13.84	17.29	20.76	25.23
1945	9.12	15.65	24.27	30.0	—
1950	10.31	18.09	26.30	—	—
1955	9.28	16.97	—	—	—

Table VII. Ratios of ectopic to intrauterine pregnancies (EP/IU ratio) in women 15 to 39 years of age in Lund, 1960-1977

Years	*Total no. of intrauterine pregnancies*	*Total no. of ectopic pregnancies*	*EP/IU ratio*
1960-64	6,143	35	1:177
1965-69	7,730	51	1:153
1970-74	9,977	70	1:144
1975-77	6,293	71	1:90

used during the period of study. In the third study,[74] 900 women proved laparoscopically to have had salpingitis between 1960 and 1974 were followed to their first pregnancy or, if not pregnant, to 1979. This study included a special analysis of ectopic pregnancy after PID. A summary of the findings on the reproductive events after salpingitis in those series will follow.

All patients had been subjected to laparoscopy to verify the diagnosis of salpingitis. The degree of the inflammatory reaction of the fallopian tubes was classified as mild, moderately severe, or severe, as defined earlier.[33] The women were followed to their first pregnancy after PID or, if not pregnant, for a mean period of 8 years. In the follow-up, the women were classified as follows:

I. *Pregnant*—women who became pregnant after (but not between) the infection(s). The first pregnancy after PID was either:
   A. *Intrauterine*
   B. *Ectopic*

II. *Not pregnant*
   A. *Voluntarily*—women who used contraceptives or who were sterilized as well as those who did not expose themselves to a risk of pregnancy (sexually inactive)
   B. *Involuntarily*—women exposed to the risk of pregnancy for more than 1 year without conceiving. The women were subclassified as either:
      1. *Post-PID infertility*—proven tubal occlusion
      2. *Other causes* or *unknown*—aspermia anovulation, endometriosis, incompletely examined, etc.

The women followed up constituted about 70% of all women treated for laparoscopically verified salpingitis between 1960 and 1974. As determined from hospital records, the women who were not followed did not differ from the 1,100 women followed up as to age, parity, marital and socioeconomic status, gonococcal/nongonococcal PID, and distribution of mild, moderately severe, and severe infection.

In the total sample, 77.1% of the women had had one infection, 17.1% two infections, and 5.8% three or more infections. Of all repeated instances of PID, 51.5% appeared within 12 months of the end of treatment for the first infection.[74]

After one or more infections, 15.2% of the women

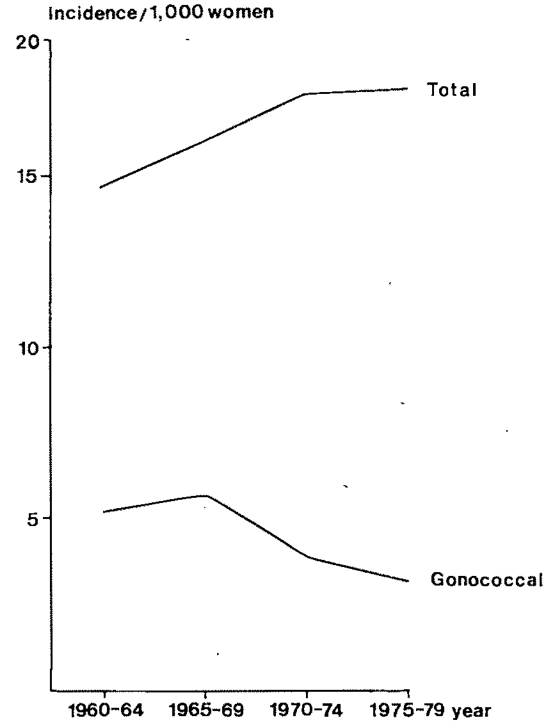

Fig. 4. Mean annual total incidence of total and gonococcal salpingitis per 1,000 women 15 to 24 years of age in Lund during the periods 1960-64, 1965-69, 1970-74, and 1975-79.

who exposed themselves to the risk of pregnancy were infertile because of tubal occlusion (Table III).[74] The postinfection infertility rate was significantly lower in the 15 to 24 year age group (13.9%) than in the 25 to 34 year age group (25.7%). The infertility rate increased significantly with the number of infections (Table IV). The difference in infertility rate between 15- to 24-year-old women and 25- to 34-year-old women was confined to those who had had only one infection. After more than one infection, no age difference in the infertility rates was observed.

In women who had had only one infection, the infertility rate increased with the severity of the inflammatory changes (Table V). In women less than 25 years of age, those who had had gonococcal-associated PID had a significantly better fertility prognosis than those who had had nongonococcal salpingitis.

If we assume an annual incidence of PID of 11 to 13 per 1,000 women among the estimated 36 million women less than 35 years of age in the United States during 1970-74 (see above),[21] we arrive at an annual mean of 400,000 to 450,000 PID cases. With an infertility rate of 15.2%, this means that more than 60,000 women are rendered infertile because of PID annually in the United States.

Using our age- and time-specific incidences for PID (Figs. 1 and 2),[23] as well as our figures for reinfections and post-PID infertility, we can calculate the cumulative numbers of women who were rendered infertile because of PID in cohorts of women born in a specific year. The results of such calculations are shown in Table VI. As can be seen, the infertility rates because of PID in cohorts of 1,000 30-year-old women born in 1940, 1945, and 1950 were 17.3, 24.3, and 26.3, respectively.

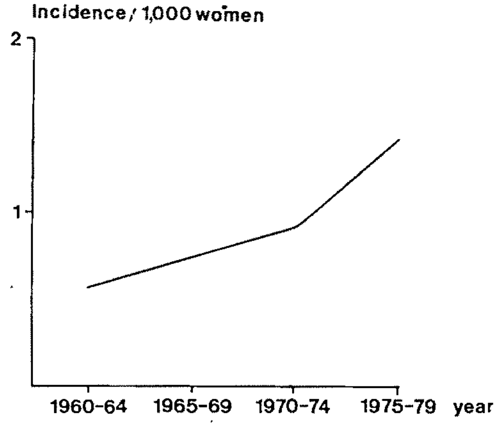

Fig. 5. Mean annual incidence of ectopic pregnancy per 1,000 women 20 to 29 years of age in Lund during the periods 1960-64, 1965-69, 1970-74, and 1975-77.

Follow-up studies of women who had had one infection and who were treated with different antibiotic regimens did not reveal any significant differences in the fertility and infertility rates.[73] The antibiotic regimens used were: streptomycin combined with penicillin, chloramphenicol combined with penicillin, ampicillin, and doxycycline.

PID and ectopic pregnancy. It has been claimed that conservative treatment of acute salpingitis has increased the frequency of ectopic pregnancies. In 1930, Holtz[illegible] reported one such pregnancy to every 200 intrauterine pregnancies after PID. In our clinic, the ratios of ectopic to intrauterine pregnancies in 15- to 39-year-old women registered in the city are listed in Table VII. There has been a relative increase in the fraction of ectopic pregnancies. The absolute number of ectopic pregnancies as measured by the annual incidence per 1,000 women increased during the period of study (Fig. 5). Out of 227 women undergoing surgery because of ectopic pregnancy and registered in the city between 1960 and 1977, 105 (46.2%) were in the post-PID state.[62]

In our prospective follow-up studies, we found a ratio of one ectopic pregnancy to every 24 intrauterine pregnancies in women treated between 1960 and 1967.[illegible] In the second study, we found a ratio of 1 : 16 in

Table VIII. Calculated reproductive events after salpingitis in cohorts of 1,000 women exposed to a chance of pregnancy

	After one infection			*After two or more infections*		
Event	*15-24 yr*	*25-34 yr*	*Total*	*15-24 yr*	*25-34 yr*	*Total*
Pregnant						
Intrauterine	827	647	737	623	568	596
Ectopic	36	78	57	87	23	55
Not pregnant						
Tubal occlusion	90	172	131	275	399	337
Other causes	47	103	75	15	10	12
Total	1,000	1,000	1,000	1,000	1,000	1,000

the *first* pregnancy after PID.[74] In a series of healthy control women who had not had PID, the corresponding ratio was 1:147.[33]

For women who have never had PID, Urquhart,[8] in a comprehensive survey of a large series of retrospective studies on the ratios of ectopic/intrauterine pregnancy, arrived at a figure of 1:300. Using the ratio of 1:300 for women who had never had PID and 1:24 for women in the post-PID state, he calculated the corresponding ratio for ectopic pregnancy per 100 woman-years of noncontraception to be 0.27 for women who never had PID and 2.7 for post-PID women (a tenfold risk increase).

Using our figures for total female population, contraceptive usage, women in the post-PID state, and number of ectopic pregnancies in women 20 to 29 years of age registered in Lund during 1970-74, we could calculate the indices of ectopic pregnancy per 100 woman-years of noncontraception to be 0.26 in women who had never had PID and 2.0 for women in the post-PID state.[62] Thus, a seven- to tenfold risk increase was found for ectopic pregnancy in women who had had PID as compared to women who had never had PID.

When analyzing the impact of women in the post-PID state on the increase in ectopic pregnancies (Fig. 5), we found that the increase in the fraction of post-PID women accounted for 24.5% of the increase in ectopic pregnancies in that specific age group.[62]

If we assume a "salpingitis cohort" of 100,000 women for a specific year, and furthermore that all women in the cohort expose themselves to a chance of pregnancy, then this single cohort will contribute at least 5,500 ectopic pregnancies unevenly distributed over the remaining years of fecundity of that specific cohort (Table VIII). This means that annually about 25,000 ectopic pregnancies in the United States are currently attributable to PID.

Combined effects of infertility and risk increase for ectopic pregnancy. Table VIII lists the calculated reproductive events after salpingitis in cohorts of 1,000 women who expose themselves to a chance of pregnancy, based on our figures. Among those women who have had one episode of PID and expose themselves to a chance of pregnancy, 12.6% in the age group 15 to 24 years and 25% in the age group 25 to 34 years will either be infertile from their PID episode or have an ectopic pregnancy. The corresponding figures after two or more infections were 36.2% and 42.2%, respectively.

The figures document the great impact that PID has on modern society. Let us illustrate by taking the 1950 cohort of women. Out of 1,000 girls born that year, today, at the age of 30 years, 138 have had one or more bouts of PID, 26 are infertile because of that infection, and nine have undergone operation because of post-PID ectopic pregnancy. Who would have dared to predict in 1950 that one out of 28 girls born during that year eventually would fail to have the children they planned, because of PID?

The conclusion of these analyses must be that ". . . pelvic inflammatory disease is . . . the most significant of all the sexually transmitted diseases in American medicine."[39]

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Salpingitis: Overview of etiology and epidemiology

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Current views of the etiology of salpingitis are widely divergent, partly because of epidemiologic and clinical differences in various studies. Of all genital pathogens, *Neisseria gonorrhoeae* has been the best documented as a cause of salpingitis, yet even the role of gonococci in salpingitis has been controversial. The proportion of salpingitis cases attributable to *N gonorrhoeae* has been influenced by contraceptive usage patterns and by the racial composition of the population studied. Gonococcal strains also may vary in propensity to cause salpingitis. For example, among white women we have found that $\text{Arg}^-\text{Hyx}^-\text{Ura}^-$ strains accounted for 32 (42%) of 76 isolates associated with salpingitis vs. 38 (72%) of 53 associated with uncomplicated gonorrhea ($p = 0.0001$). There is little doubt *Chlamydia trachomatis* also causes salpingitis, although the role of chlamydiae is uncertain in culture-negative patients who have high serum antibody titers against this agent. Other organisms, including facultative pathogens, such as coliforms, *Haemophilus influenzae,* groups B and D streptococci, and *Mycoplasma hominis*, and anaerobes, such as peptococci, peptostreptococci, and *Bacteroides* species, often have been implicated, particularly in recurrent or severe salpingitis or in intrauterine device–associated cases, usually but not always in the absence of gonococcal infection. Comprehensive microbiologic study of tuboperitoneal specimens obtained at laparoscopy or laparotomy must be combined with comprehensive serologic studies in patients with salpingitis who are well characterized clinically and epidemiologically in order to resolve which organisms are involved, in what combination, and in which type of patient. (AM J. OBSTET. GYNECOL. 138:893, 1980.)

WE HAVE BEEN invited to review some of the important concepts that have emerged from recent studies of the epidemiology and etiology of salpingitis, and to interpret and speculate on the implications of differences in the results of these studies. This article focuses on selected areas of controversy, with no attempt to be comprehensive.

The routine use of laparoscopy by Jacobsen and Weström[1] for the objective diagnosis of salpingitis set guidelines for subsequent studies of salpingitis and demonstrated the inaccuracy of clinical diagnosis. This central problem, together with marked geographic differences and temporal shifts in the incidence of gonococcal and chlamydial infections and in contraceptive usage patterns and other factors, have contributed to widely divergent views of the causes of salpingitis. For example, some investigators speculate that gonococci cause nearly all salpingitis, while others believe gonococci cause less than 10% of salpingitis; some American investigators believe anaerobes contribute to the pathogenesis of most salpingitis, while the Swedish studies implicate anaerobes in almost no cases of salpingitis; chlamydia are implicated as causing 60% of salpingitis cases in Lund and none of the cases in San Francisco. We would therefore like to review the evidence for each of these various infectious agents as pathogens causing salpingitis, and offer some hypotheses on how clinical and epidemiologic differences between different studies may help to account for some of the divergent results that have been obtained concerning the etiology of salpingitis.

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Supported by Grant AI 12192 from The National Institute of Allergy and Infectious Diseases, and by The Division of Hospitals and Clinics, Bureau of Medical Services, Public Health Service Project Grant SEA 77-06-72.

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Chlamydial salpingitis

Chlamydia trachomatis was first isolated from the fallopian tubes of women with salpingitis separately by two groups in Sweden.[2, 3] Mårdh and associates[3] recovered *C. trachomatis* from the endocervices of 36% of women with salpingitis, and from the fallopian tubes of six of seven in whom *C. trachomatis* was recovered from the endocervix. Subsequently Treharne and colleagues[4] showed an IgG microimmunofluorescent (micro-IF) antibody titer ≥ 1:64 against *C. trachomatis* in 62% of women with salpingitis as compared to only 11% of controls. The geometric mean titer (GMT) of antibody was proportional to the severity of salpingitis at laparoscopy. Ripa and co-workers[5] have produced experimental salpingitis in monkeys with *C. trachomatis.*

There seems little doubt that *C. trachomatis* causes salpingitis. However, the exact proportion of salpingitis caused by chlamydia in various studies, and the role of chlamydia in culture-negative patients who have IgG antibody titers ≥ 1:64, are uncertain. IgM antibody was found in only 23% of cases studied by Treharne and associates and the GMT of IgM antibody was not correlated with severity of salpingitis at laparoscopy. The number of controls in the Swedish studies was small, and their comparability to patients with salpingitis was not clear.

We previously have isolated *C. trachomatis* from the cervices of identical percentages of women, seen in our clinics for sexually transmitted disease (STD), who do and do not have salpingitis; and among those with salpingitis, the same percentage of those with and without gonorrhea have positive cervical cultures for chlamydia—about 20%.[6] In a limited analysis of our data, the GMT of micro-IF antibody was only slightly higher among[7] women with salpingitis (1:138) than among matched female patients without salpingitis (1:103).[6, 8] However, we have noted that only five of 15 patients with salpingitis who demonstrated a fourfold or greater rise in micro-IF antibody titer had positive cervical cultures for *C. trachomatis,* suggesting insensitivity of cervical cultures in salpingitis.[6]

If women with high GMT of antibody but negative cultures actually have chlamydial salpingitis, then confirmatory evidence could be obtained as follows: (1) recovery of *C. trachomatis* from the urethra of the sex partner—a study not yet attempted; (2) isolation of *C. trachomatis* from the urethra or other site in the patient; (3) demonstration that these cases clinically and epidemiologically resemble each other and chlamydial salpingitis (as proven by culture), rather than some other type of salpingitis; (4) demonstration of significantly lower prevalence of IgG antibody titers ≥ 1:64 in women with salpingitis caused by other agents as proven by laparoscopic culture; and (5) demonstration of significantly lower prevalence of IgG antibody titers ≥ 1:64 in matched controls without salpingitis.

In man, urethral infections with chlamydia are clinically less severe than gonococcal urethritis. Similarly, Svensson and associates[9] have found that salpingitis associated with *C. trachomatis* infection (as determined by isolation of the agent or by serology) is clinically more indolent, with a longer mean duration of pain and less prevalence of fever in comparison with salpingitis associated with gonorrhea. However, the severity of disease, as determined by laparoscopic findings, for these two forms of salpingitis was similar. If salpingitis cases included in the Swedish studies have been clinically less acute and less severe than those in the United States studies published to date, which have tended to include what might be termed "emergency room" salpingitis, then this might help explain the lower proportion of cases in the United States that have been chlamydia associated and the higher proportion that have been gonorrhea associated. The relative risks of salpingitis with gonococcal vs. chlamydial infections might be compared by examining the prevalence of clinical evidence of salpingitis among gynecology or female STD clinic patients who have either chlamydial infection or gonococcal infection, or both, or neither. However, even if the prevalence of salpingitis in individual patients with *C. trachomatis* infection turns out to be lower than in those with gonococcal infection, the total incidence of chlamydial salpingitis could, nonetheless equal or exceed the incidence of gonococcal salpingitis in industrialized countries, where chlamydial infection probably is much more common than gonococcal infection in women.

There is further evidence that *Chlamydia trachomatis* may cause two syndromes related to salpingitis—postpartum endometritis[10] and the Fitz-Hugh–Curtis syndrome (perihepatitis).[8] Furthermore, growing evidence links *C. trachomatis* infection to infertility caused by bilateral tubal occlusion.[11, 12] Punnonen and colleagues[11] have shown a higher prevalence of chlamydial antibody among infertile women than among pregnant controls; and among infertile women, they showed a higher titer of antibody among those with tubal occlusion than among those with normal hysterosalpingograms.

Tubal damage and peritonitis in chlamydial salpingitis may involve immunopathologic mechanisms. This is suggested by the absence of cytotoxicity of *C. trachomatis* in tubal organ culture[13] (in contrast with the toxicity of *Neisseria gonorrhoeae* in organ culture),[14] by the correlation of severity of tubal damage with GMT

Table I. Number of Arg$^-$Hyx$^-$Ura$^-$ isolates per total number of isolates (percent) among black and white Seattle women with gonorrhea, with or without salpingitis

Year	*Black*		*White*		
	Salpingitis	*Uncomplicated*	*Salpingitis*	*Uncomplicated*	
1972	1/12 (8%)	3/8 (38%)	8/18 (45%)	12/14 (86%)	p = 0.03
1974-75	5/22 (23%)	1/7 (14%)	6/16 (38%)	20/28 (71%)	p = 0.05
1977-78	10/35 (29%)	18/42 (43%)	3/8 (38%)	6/11 (55%)	p = 0.52
Totals	16/69 (23%)	7/23 (30%)	32/76 (42%)	38/53 (72%)	p = 0.001

of IgG antibody to chlamydia,[4] by the absence of IgM antibody in most cases,[4, 8] by the limited histopathologic studies done in experimental infections,[5] and perhaps by analogy with trachoma. In Fitz-Hugh–Curtis syndrome in particular, serologic data suggest that this particular complication may more likely be associated with heterologous reinfection than with the first infection by *C. trachomatis.*[8] Studies of experimental infections in intact animals may therefore be particularly important in analyzing the pathogenesis of *C. trachomatis* salpingitis. In addition, further light may be shed on this question by studies of the cellular immune response in man and further serologic classification of human cases of *C. trachomatis* infections with and without salpingitis as primary infection vs. homologous reactivation/reinfection vs. heterologous reinfection.

Gonococcal salpingitis

Neisseria gonorrhoeae has been isolated from the cervices of anywhere from 10% to 70% of women with salpingitis, in recent studies.[15, 16] Even among those with positive cervical cultures, the proportion who have had positive tubal or peritoneal cultures has ranged from 8% to 70%.[15, 16] Those who found gonococci least often have questioned its importance in salpingitis. Some have implicated chlamydia as the main cause of salpingitis,[4] while others have suggested that gonococci merely pave the way for invasion of the fallopian tubes by mixed flora, particularly anaerobes.[17–19]

Where is the common ground in these apparently contradictory observations? New concepts sometimes can emerge from contradiction. Some epidemiologic and clinical observations may help explain some of these differences.

Host factors in gonococcal salpingitis. Race has been correlated not only with risk of gonorrhea, but also with risk of salpingitis among women with gonorrhea. Nonwhite women represented 46% of all women with gonococcal salpingitis vs. only 29% of women with uncomplicated gonorrhea in Seattle.[7] This apparently higher risk of black women developing salpingitis with gonorrhea has not yet been explained, but differences in the racial composition or socioeconomic status of the populations studied could account for some of the observed differences in the proportion of salpingitis cases associated with gonorrhea, particularly in those studies done in Europe vs. those conducted in the United States.

Since case-control studies suggest that oral contraceptive usage is associated with a decreased risk of salpingitis among women with gonorrhea,[7, 20] it is quite possible that trends in use of oral contraceptives by women might influence the proportion of gonococcal infections which lead to salpingitis. Thus the declining rate of oral contraceptive usage, which has been seen in the United States since 1974,[21] might tend to lead to an increased risk of salpingitis among women who acquire gonorrhea.

Gonococcal virulence as a factor in salpingitis. Sackel and his co-workers[22] found that gonococci from women with salpingitis were more resistant to penicillin than those from women with uncomplicated gonorrhea. We have found that certain auxotypes of gonococci are more likely than others to be associated with salpingitis. Since auxotype is strongly correlated with race,[23] as is the risk of salpingitis in women with gonorrhea, it is essential to control for race in such an analysis. As shown in Table I, we have found that Arg$^-$Hyx$^-$Ura$^-$ strains of *N. gonorrhoeae* accounted for only 48 (33%) of 145 isolates from women with salpingitis, compared to 45 (59%) of 76 isolates from women with uncomplicated gonorrhea (p = 0.0002). Among white women with gonorrhea, Arg$^-$Hyx$^-$Ura$^-$ strains accounted for 32 (42%) of 76 isolates from women with salpingitis vs. 38 (72%) of 53 with uncomplicated gonorrhea (p = 0.0001). These data are consistent with those previously published by Sackel and colleagues,[22] since Arg$^-$Hyx$^-$Ura$^-$ isolates are more sensitive than other auxotypes to penicillin. It appears that some strains of gonococci are more likely than others to be associated with salpingitis. We have reported previously that the proportion of gonococcal isolates which are of the Arg$^-$Hyx$^-$Ura$^-$ strain is much lower for black women than for white women.[23] Thus the higher risk of salpingitis among black women with gonorrhea might be explained partly by the higher proportion of gonococcal infections in black women caused by strains that are more likely to produce salpingitis.

It is possible that trends in the proportion of $Arg^-Hyx^-Ura^-$ gonococci might be reflected in trends in the proportion of gonococcal infections that are complicated by salpingitis. Weström[20] found that the incidence of gonorrhea in Lund, Sweden, rose during the 1960s and early 1970s, despite a stable rate of gonococcal salpingitis. The proportion of cases of gonorrhea complicated by gonococcal salpingitis apparently fell in Lund during that period of time. A similar phenomenon has been observed in Örebro, Sweden.[24] Thus it is of interest that among strains of gonococci collected and stored at the Statens Seruminstitut in Copenhagen from the early 1940s to the present, the proportion of the $Arg^-Hyx^-Ura^-$ strain rose progressively during the 1960s and early 1970s,[23] a finding consistent with the declining proportion of gonococcal infections complicated by salpingitis in Sweden during that same period.

Polymicrobial salpingitis

We initially used the term "polymicrobial etiology" of pelvic inflammatory disease[6] to indicate that in the general population there are many agents capable of causing salpingitis, as well as to indicate that in the individual patient more than one agent could be involved.

Possible role of gonococci in polymicrobial salpingitis. The view that gonococci pave the way for anaerobic invasion of the fallopian tubes was put forth most recently by Chow and co-authors[17] and was espoused to some extent by others,[18, 19] who usually found other flora present with gonococci in culdocentesis fluids from patients with salpingitis. It seems clear from several American studies,[6, 18, 19, 25, 26] especially one employing routine laparoscopy,[26] that a certain number of the cases of salpingitis with cervical gonorrhea seen in the United States are associated with polymicrobial tuboperitoneal infection, but the proportion probably is not large.

Among women with "severe" salpingitis, we found bacteria other than gonococci in peritoneal exudate from two of 16 with cervical gonorrhea and from 14 of 22 without cervical gonorrhea ($p < 0.025$).[6] Thus, we believe that although polymicrobial tuboperitoneal infection can be found in women with gonorrhea, it is much more common in women without gonorrhea. For the most part we have been unable to find serologic or epidemiologic evidence of recent gonococcal infection among women who have negative cervical cultures for *N. gonorrhoeae* and tuboperitoneal infection with organisms other than *N. gonorrhoeae*.

Clinical and epidemiologic variables, as well as variations in methods of obtaining peritoneal specimens, also help to explain variations in the proportion of salpingitis cases associated with mixed tuboperitoneal infections. The proportion of salpingitis cases associated with tuboperitoneal infection by agents other than *Neisseria gonorhoeae* increases with increasing duration and severity of symptoms.[6] This suggests that gonococcal salpingitis might lead to superinfection by other organisms if the disease is allowed to progress unchecked, without treatment. These other organisms might gain access to the upper genital tract at the same time as the gonococci, or soon after, presumably by the transluminal route. Certain organisms might even suppress the growth of *N. gonorrhoeae*, preventing its recovery. Alternatively, it appears that some organisms which cause primary nongonococcal salpingitis (e.g., *Chlamydia trachomatis*) produce a more indolent infection that can be tolerated longer than gonococcal salpingitis before treatment is sought.[9]

Sweet and his group[26] reported that the proportion of patients with nongonococcal tubal or peritoneal infection is greater for recurrent salpingitis than for first attacks. We previously reported isolating *N. gonorrhoeae* from the endocervices of 69 (49%) of 140 women with a first attack of salpingitis vs. 22 (34%) of 64 with a recurrent attack ($p = 0.05$, Fisher's exact test).[27] It may be that the initial episode of gonococcal salpingitis leads to tubal damage and increased tubal susceptibility to delayed subsequent infection by other organisms that ordinarily are less "tubotropic." Also, there is some evidence that one attack of gonococcal salpingitis protects against subsequent attacks of gonococcal salpingitis by antigenically related strains[28]; it follows that an increased proportion of subsequent cases of salpingitis would be nongonococcal. The term "secondary" pelvic infection conventionally refers to postobstetric or postsurgical infections of the pelvis by components of the vaginal flora. In addition, certain components of the vaginal flora might produce "secondary" invasion of the fallopian tubes either during an attack of primary gonococcal salpingitis or during a subsequent attack of salpingitis involving tubes previously damaged by gonococcal salpingitis. It would be extremely interesting to know whether *C. trachomatis* or *Mycoplasma hominis* infection might also predispose to such secondary invasion.

Mixed infection with anaerobic and/or facultative bacteria other than *N. gonorrhoeae* or *C. trachomatis*. Because of the long-overdue growth of the subspecialty of anaerobic microbiology, with no counterbalancing group having a special interest in "aerobic microbiology" per se, the pendulum has swung toward emphasis on the importance of anaerobes in salpingitis. However, the bacteria isolated by culdocentesis or laparoscopy from patients with salpingitis actually include both anaerobic and facultative bacteria, which have been isolated equally often, sometimes with mixed anaerobic-facultative infections, sometimes with anaerobic infection alone, sometimes with facultative infection alone.[6, 17–19, 25, 26] The predominant facultative pathogens found in tuboperitoneal fluids from women with salpingitis have included coliforms, *Haemophilus influenzae* group B, groups B and D streptococci, and *M. hominis*. Prominent anaerobic pathogens found have included peptococci, peptostreptococci, and *Bacteroides* species. The question of which is the predominant *Bacteroides* sp. in acute salpingitis has excited some controversy. *B. melaninogenicus* was the predominant *Bacteroides* sp. in some studies,[25] and *B. fragilis* was predominant in others.[6, 19, 29] *B. fragilis* often is involved in "secondary" pelvic infections in women (e.g., postsurgical infections), but is not a prominent member of the vaginal flora, suggesting this organism is relatively more virulent than the more common vaginal Bacteroides sp., such as *B. capillosus*, *B. bivius*, and *B. disiens*. In some studies[17, 25, 26] *B. fragilis* was not isolated from tuboperitoneal cultures from any of the patients with salpingitis. The serum antibody response to *B. fragilis* capsular polysaccharide antigen has correlated with isolation of *B. fragilis* in our own studies, supporting the etiologic significance of this agent.[30] A serologic response to *B. fragilis* also was apparent in some cases of salpingitis studied by Paavonen,[31] but in only two of eight patients from whom *B. fragilis* was isolated in Sweden.[29]

***M. hominis* and salpingitis.** The role of *M. hominis* has been supported by similar serologic studies[32] and by isolation of *M. hominis* from tuboperitoneal cultures and by laparoscopy in some cases.[33] It is of interest that experimental infection of grivet monkeys with *M. hominis* produced parametritis as well as tubal inflammation.[34] This might suggest a parametrial rather than luminal route of infection. Others occasionally have recovered *M. hominis* from the tubes or cul-de-sac of patients with salpingitis, but the role or this sexually transmitted agent in primary acute salpingitis is less well established than that of *N. gonorrhoeae* or *C. trachomatis*. The role of *M. hominis* as a primary cause of salpingitis would be more convincingly established if it could be shown that cases of *M. hominis*–associated salpingitis are clinically unique, for example, uniquely associated with parametritis in man, as in grivet monkeys.

Use of intrauterine device (IUD) and polymicrobial vaginitis. It is well established that IUD usage is associated with an increased risk of salpingitis, particularly nongonococcal salpingitis. For example, we have reported isolation of bacteria other than *Neisseria gonorrhoeae* from the peritoneal exudate of nine (47%) of 19 IUD users and six (25%) of 24 nonusers ($p = 0.20$).[6, 20] IUD usage has been found to be associated with endometrial bacterial colonization,[35] and it is likely that the IUD tail facilitates the ascent of bacteria from the vagina to the endometrial cavity. It is possible that the IUD also alters the vaginal flora itself. We have found that IUD users are significantly more likely than nonusers to have nonspecific vaginitis,[36] a condition associated with vaginal overgrowth by anaerobic bacteria.[37] Thus, IUD usage may pomote development of nongonococcal salpingitis by more than one mechanism.

It seems likely that the lower prevalence of anaerobic or polymicrobial infection in cases of salpingitis in Sweden, as compared to cases in the United States, is related to differences in severity of infection or in duration of disease before diagnostic steps are undertaken. It also is conceivable that gonococcal salpingitis is more likely than other forms of primary salpingitis (e.g., chlamydial salpingitis) to lead to secondary infection by vaginal flora, and that gonococcal salpingitis is more common than chlamydial salpingitis in populations that have been studied in the United States than in Sweden. It is even possible that in populations studied in the United States the vaginal flora is more likely to cause secondary tubal infection (e.g., the average concentration of vaginal anaerobes could be higher) than in the populations studied in Sweden.

It seems clear that comprehensive microbiologic study of tuboperitoneal specimens obtained at laparoscopy or laparotomy must be combined with comprehensive serologic studies in patients with salpingitis who are well characterized clinically and epidemiologically, to resolve the issue of which anaerobes or facultative organisms are involved and in which type of patient.

Other epidemiologic clues to the etiology and pathogenesis of salpingitis. (1) One consistent observation is that the onset of salpingitis associated with gonorrhea is correlated with the first 2 weeks of the menstrual cycle—a finding we have reconfirmed.[7] A more recent, possibly related, observation made by Weström[20] is that the annual incidence of salpingitis among sexually active women is many times higher among 15- to 16-year-old teenagers than among women over 20 years of age. The latter observation might be explained by the fact that women who become sexually active at age 15 to 16 years have more partners than those who become active later. Another possibility, suggested by Drs. Tom Bell and John James,* is that the cervical mucus in very young, sexually active women may still be estrogen dominated, which might permit it to be more readily penetrated by gonococci or sperm having gonococci or other bacteria attached. The same explanation might account for the onset of gonococcus-associated salpingitis during the estrogenic phase of the cycle, and the converse might explain partly the decreased risk of gonococcal salpingitis in users of oral contraceptives who have more dense cervical mucus.

(2) A striking conviction of the Swedish investigators is that primary salpingitis can rarely be confirmed laparoscopically in women who do not have increased leukocytes in vaginal fluid and/or a proven sexually transmitted infection. Presumably, these leukocytes emanate from the cervical os as a reflection of cervicitis or inflammation above the cervix. Although conventional dogma now holds that cervical culture results do not correlate with tubal culture results, one wonders whether more careful examination by Gram stain and direct microscopy and quantitative culture of material obtained directly from the endocervix after it has been carefully cleansed might not show a closer correlation of endocervical flora with tuboperitoneal flora than previous studies have suggested. This would be somewhat analogous to the clinical concept that transtracheal aspirates may serve better than expectorated sputums for the culture diagnosis of pneumonia, and that the microscopic examination of purulent areas of Gram-stained expectorated sputum may be more informative than nonquantitative culture of expectorated sputum, and may help determine whether the specimen is contaminated by oral epithelial cells and associated flora.

Discussion

In summary, the different studies of the infectious cause of salpingitis in markedly different settings have produced markedly different results, which can be reconciled only partly, but perhaps need not be reconciled completely in view of the many factors that might influence the etiology of salpingitis. These include the widely varying prevalence of gonorrhea and nonuniform distribution of gonococcal types by region and within population subgroups, varying epidemiology of *C. trachomatis* infections, varying usage of IUD and oral contraceptives, as well as the influence of age, race, parity, duration and severity of symptoms before seeking care (which may be related to accessibility of health care), accuracy of diagnosis, and the many different methods used to study etiology. The central and obvious theme of recent studies is that the etiology of salpingitis is polymicrobial; salpingitis in general has multiple causes, and individual cases may have multiple causes. This is hardly surprising. Why should salpingitis be different from upper or lower respiratory or other abdominal infections in this regard? It is essential to carefully define the populations studied, both epidemiologically and clinically, and the methods used to study them, to eventually allow data from different centers to be compared. Accurate diagnosis of salpingitis is most essential.

Among the many practical questions which proceed from the epidemiologic data collected so far are the following 10:

1. Can we accumulate historical and seroepidemiologic data directly on the infectious etiology of the complications of salpingitis in the United States, such as ectopic pregnancy, infertility, and chronic pelvic pain, through case-control studies as an alternative to the far more difficult cohort studies that have been so productive in Sweden, but which would be far more difficult here?
2. What practical methods can be developed to improve clinical diagnosis of salpingitis for optimal studies of the infectious etiology?
3. What are the overall therapeutic implications of our current concepts of the multiple causes of salpingitis? Can more practical methods be devised to distinguish which microbial cause is implicated in the individual patient with salpingitis, as a guide to therapy in that patient?
4. In view of the correlation of certain gonococcal auxotypes with risk of gonococcal salpingitis, what are the strain-specific pathogenic mechanisms responsible?
5. In view of the evidence that natural gonococcal infection provides type-specific protection against recurrent gonococcal salpingitis, what are the implications for development and deployment of gonococcal vaccine?
6. If *Chlamydia trachomatis* infection causes salpingitis by immunopathologic mechanisms, what are the mechanisms? How can tubal scarring be prevented by therapy? What are the implications for chlamydial vaccine?
7. What is the need for treatment of sex partners of women with nongonococcal salpingitis? Is asymptomatic infection of the man with *C. trachomatis*, *M. hominis*, or other flora a cause of recurrent salpingitis in some women?

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*Personal communication.

8. Can an IUD be designed that does not lead to anaerobic infection?

9. Would routine, periodic examination of IUD users for vaginal anerobes identify those women at risk of developing IUD-related salpingitis? Would a brief course of appropriate antimicrobial prophylaxis to suppress vaginal anaerobic growth protect her?

10. In a woman recovering from salpingitis, IUD contraception seems risky; would oral contraceptive usage for a 1 to 3 year period thereafter, during which the risk of salpingitis is greatest, protect her from recurrent salpingitis? Would oral contraceptives also protect adolescents who become sexually active and are at the highest risk?

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Trends for gonorrhea and pelvic inflammatory disease in England and Wales and for gonorrhea in a defined population

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Recent trends for gonorrhea and pelvic inflammatory disease in England and Wales are presented using routinely available data. The drawbacks of these data are examined, and a study is described which has attempted to rectify some of these inadequacies. The study attempted to identify patients with and without symptoms of gonorrhea, trichomoniasis, and candidiasis, living in a defined population. In addition, a calculation has been made of the number of cases of PID treated on an ambulatory basis in clinics for sexually transmitted disease in England and Wales. (AM. J. OBSTET. GYNECOL. 138:901, 1980.)

AN ORGANIZED and formal service for the treatment of sexually transmitted diseases (STDs) was created originally in the United Kingdom in 1916, following the Royal Commission on Venereal Diseases. This service traditionally has been based in hospitals and clinics specializing in the STDs. Physicians in charge of clinics in England, Wales, Scotland, and Northern Ireland are required to submit reports every 3 months to their Chief Medical Officers of the number of cases seen in their clinics. This article examines the trends for gonorrhea and pelvic inflammatory disease in England and Wales. These two countries contain 82% of all the clinics in the United Kingdom and account for 93% of all the cases seen in the clinics within the Kingdom.

Because of limitations in reported data, special studies have been carried out from time to time. Previous research has been in the nature of ad hoc screening surveys within certain clinical settings, such as antenatal and gynecology clinics. Patients who have sought care are sampled without any reference to a population at risk and, thus, a stable denominator.[1-5] My colleagues and I felt it was necessary to carry out a survey that measured both symptomatic and asymptomatic disease in a defined population to obtain data representative of all those at risk.

Methods

Trends for gonorrhea. Reported cases of gonococcal infection during a 12 year period (1966 through 1977) were examined to determine incidence trends. Incidence rates by sex and age groups were calculated per 100,000 population. There are two limitations inherent in the notification process. First, the figures relate to number of cases, not patients, so that a patient can reappear several times in the published statistics for a year. This can occur because a patient has more than one disease diagnosed at the same point in time, patients may contract one or more diseases on several separate occasions during the year, and finally, in many instances, there may be difficulty in differentiating reinfection from relapse. The second major limitation is that STD clinics differ in their criteria for establishing a diagnosis and reporting diseases. Past investigation into clinic practices throughout England and Wales has highlighted some of these variations[6, 7] and a further study to quantify patients as opposed to cases treated in clinics has been completed recently.

Trends for PID. The number of patients with PID admitted to hospitals in England and Wales can be derived from the Hospital In-Patient Enquiry (HIPE). This is a 10% sample of all hospital deaths and discharges. Similar to the clinic returns, the HIPE records cases, not patients, and there are no criteria for the establishment of a diagnosis of PID except that it is the physician's final diagnosis. Thus some patients will be reported who have many clinical features of the disease, plus laparoscopic and genital tests. Other patients will have undergone no confirmatory tests and will

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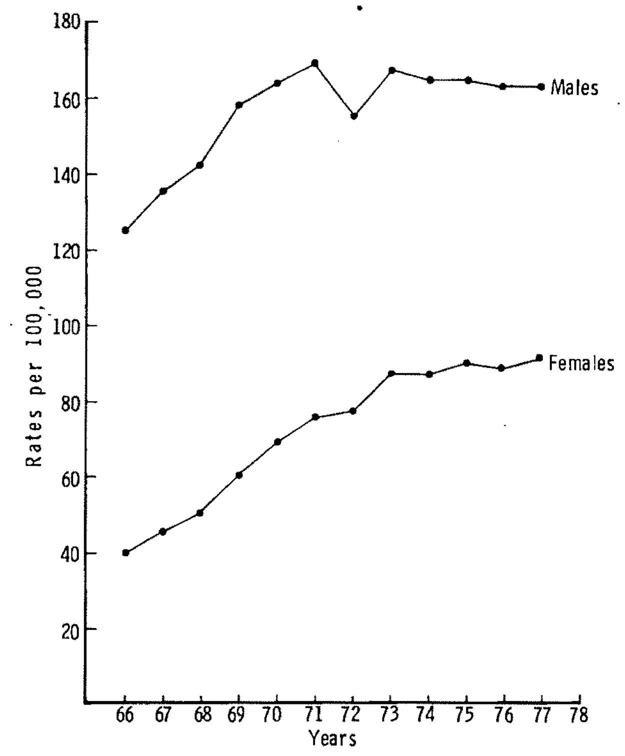

Fig. 1. Gonorrhea (postpubertal) rates per 100,000 population (England and Wales) in men and women.

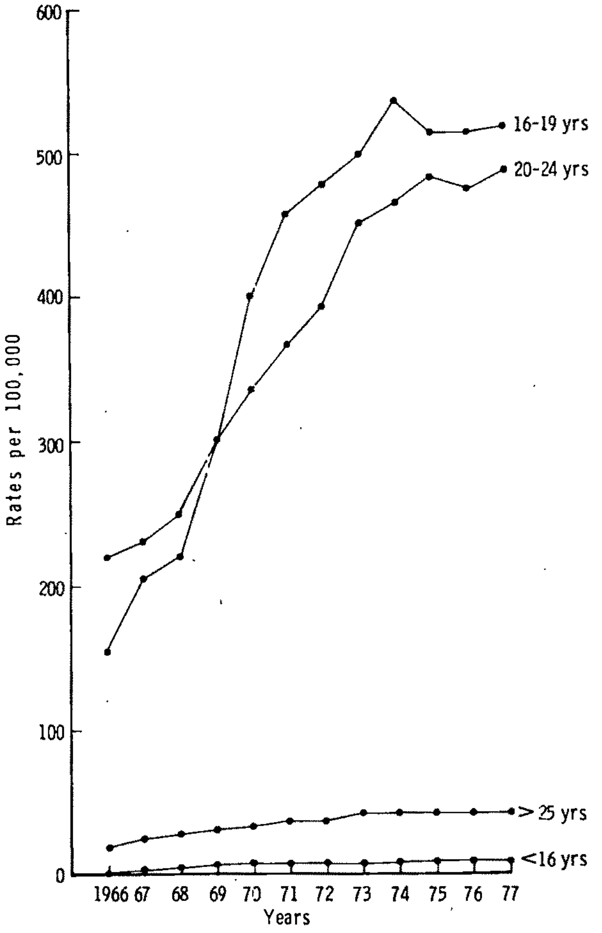

Fig. 2. Gonorrhea rates per 100,000 female population by age groups (England and Wales).

have received empirical treatment. A successful response to therapy has confirmed the diagnosis in the physician's eyes.

These data from HIPE are published on an annual basis by the Office of Population Censuses and Surveys (OPCS). Unfortunately, the routinely published categories used in HIPE are not entirely satisfactory for the purposes of identifying specific cases of acute PID. These categories are "salpingitis-oophoritis and cervicitis" and "other female genital disorders." The OPCS has allowed me access to the raw data. All the "true" cases of acute PID were identified in the original data as "acute salpingitis and oophoritis," "unspecified salpingitis and oophoritis," and "pelvic inflammatory disease." Virtually all the cases in the last two categories are acute-onset illnesses, even though at first glance these disease categories appear to be vague and ill defined.

Special prevalence study. There are two major reasons why gonorrhea and other STDs may be underrecorded. First, figures do not take into account patients treated outside STD clinics by family or private practitioners and in antenatal and gynecology clinics; second, there is no routine way of calculating the number of asymptomatic patients who never seek care. In view of this underrecording and limitations in the available data, a prevalence study in a defined population was carried out. An attempt was made to identify patients with and without symptoms, suffering from gonorrhea, trichomoniasis, candidiasis, and potential complications such as PID.

The study area was defined as the administrative Health Area of Brent and Harrow. This area is situated in North West London and contains a broad cross section of social and ethnic groupings with a total of 485,883 inhabitants. From census data, it was possible to identify the number of women in different age groups.

Women aged 15 to 60 years were included in the study when they attended the three hospitals in the area providing antenatal and gynecologic care. We sampled three family planning clinics within the study area, chosen to be representative of the different geographic and demographic characteristics known to exist in the area. The number of cases of STD that could be treated by general practitioners without refer-

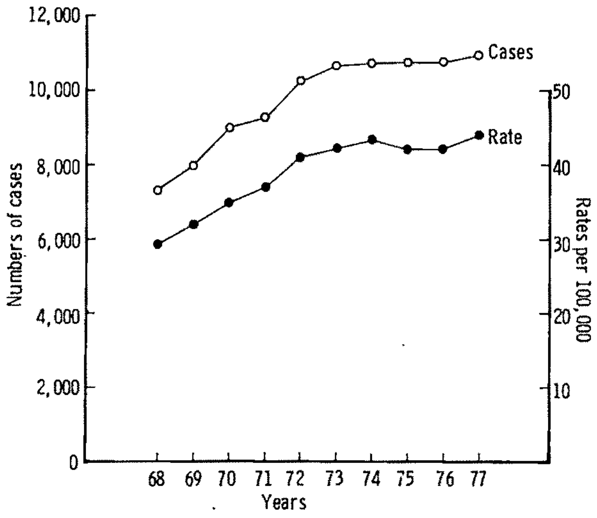

Fig. 3. Number of cases and discharge rates for acute, unspecified salpingitis and PID (England and Wales).

ral were quantified. A sample of 17 general practitioners was drawn and stratified by size of practice and whether or not the doctors carried out family planning. The practitioners were asked to record patients' symptoms, the results of genital examinations and bacteriologic tests if carried out, and treatment administered, if any.

Because of the asymptomatic nature of the diseases under investigation and the need to obtain information on those patients who do not consult doctors, I did not wish to limit sampling and examination only to women seeking care from their doctors. Further data from one physician's general practice was included in this study. All women aged 16 to 60 years (2,000) registered with the practice were identified, and half were invited to undergo an examination. It was emphasized that all women should come forward whether or not they had symptoms.

Even though a number of different agencies were used, collection of data and examinations were standardized. All women were asked to complete a self-administered questionnaire that recorded demographic data and information concerning present and past genital symptoms and their treatment, if any. Two research nurses were responsible for the genital examinations. Cultures were taken from appropriate sites for *Neisseria gonorrhoeae, Trichomonas vaginalis,* and *Candida albicans.*

Results

Gonorrhea trends. The trends for gonorrhea in England and Wales are shown in Fig. 1. Rates per 100,000 population for men and women were calculated over

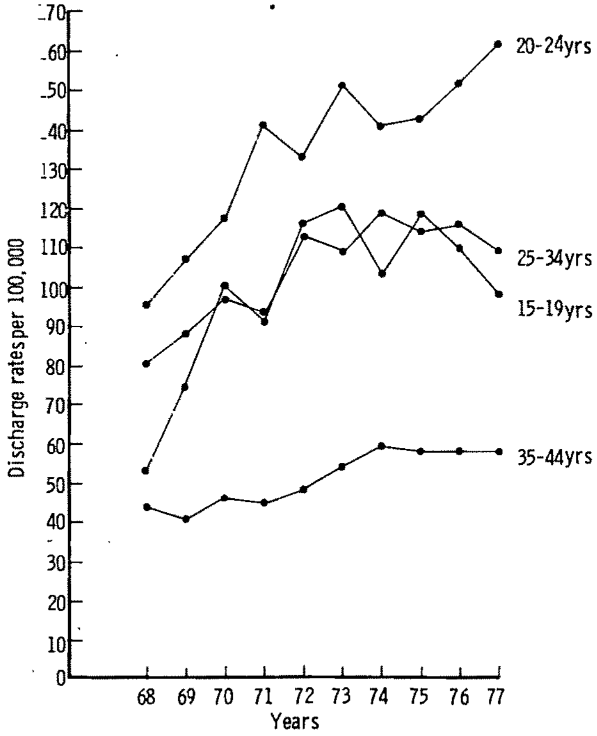

Fig. 4. Rates of acute, unspecified salpingitis and PID per 100 000 population by age groups (England and Wales).

the most recent 12 year period for which figures are available. The rates for both sexes have shown a steady increase (more marked in women) from 1966 to 1977. These increases appear, however, to have leveled off recently. In 1977, the rates represented 37,831 and 22,275 cases in men and women, respectively. Despite the leveling off over the last few years, there has been an increase of 31% for men from 1966 to 1977 and 130% for women over the same time period. Broken down by different age groups the rates give a clearer picture of those experiencing the greatest increase. Fig. 2 illustrates gonorrhea rates for women by age over the same time period. The major increases have been in women aged 16 to 19 years and 20 to 24 years.

Trends in PID. Fig. 3 reveals the trends in the number of cases and rates of acute salpingitis over a 10 year period. From 1968 to 1977, both the number of cases and the rate have increased by 50%. The number of cases requiring hospitalization in 1977 totaled 10,960 and represents 92,064 hospital bed-days. The current cost of an acute hospital bed in England and Wales is £30 ($70.41) per day.[8] Thus, the cost of all hospitalization in cases of salpingitis per annum is £2,761,920 ($6,481,859). This figure includes both direct and indirect costs. Fig. 4 indicates that the age-specific PID rates are similar to the female age-specific rates for gonorrhea with major increases occurring in the 15 to 19 and 20 to 24 year age groups.

Special prevalence study. The results of the study are preliminary at this stage, and it will be necessary to carry out some corrections and weighting of the data. Once this is done, it will be possible to quantify the cases of disease that are being treated or recognized in other settings apart from STD clinics. Nevertheless, a total of 2,286 women were screened in the special examination of asymptomatic women. Altogether, 228 (10%) were found to have candidiasis, 97 (4%) trichomoniasis, and five (0.2%) gonorrhea.

The screening concentrated on those who had not sought care for STD. However, some women residing in the study area sought care for disease either within or outside the defined area. Information about such cases was obtained from the one clinic within the study area and all the other clinics outside the area. After this exercise, a further 20 cases of gonorrhea were found, totaling 25 cases.

Discussion

Similar trends for gonorrhea and PID are seen in most other developed countries. It has been indicated that even though routine sources of data are available in England and Wales, they are undoubtedly an under estimate of the total gonorrhea problem in the community. PID, on the other hand, is underrecorded since patients can be treated on an ambulatory basis. The routine figures returned to OPCS relate only to hospitalized cases. Physicians in STD clinics do not record cases of PID as a separate item. Other physicians who see outpatients with PID are general practitioners, gynecologists, and physicians working in the STD clinics. The end result is underrecording of PID cases. The Middlesex Hospital is the second largest clinic in the United Kingdom, and in 1978 a total of 21,000 cases of STD and other diseases were seen. Of these, 1,758 were cases of gonorrhea and nonspecific genital infection in women. In addition, 85 cases of PID were seen, just under 5% of the total cases of gonorrhea and nonspecific infection, and seven of the patients were admitted; only 8% of those had salpingitis.

The total number of cases of PID requiring hospitalization in England and Wales recorded and appearing in HIPE is 10,960. Other physicians and clinics will have different management policies and case mixes. However, if the ratio of ambulatory/hospitalized cases of PID is similar to that in clinical experience at the Middlesex Hospital, then the actual number of cases of PID far exceeds the cases reported in HIPE.

Our special prevalence study is not complete at present. However, even at this stage, it seems clear that routine screening of all antenatal, gynecologic, and family planning clinics and of sexually active women consulting general practitioners is not the way of controlling gonorrhea in this country. Also, there is no evidence to suppose a significant number of cases of untreated gonococcal salpingitis would be detected. Only five cases of untreated gonorrhea were identified in our study.

England and Wales possess an excellent STD clinic service with a sophisticated recording system for cases seen in clinics and patients hospitalized with the complications of the diseases. Despite this, the data systems can be improved. At present, these systems indicate only part of the total clinical picture of the STDs. Accurate comprehensive figures are essential for monitoring changes in the STDs and for planning appropriate medical and other facilities required for clinical care and control.

I am grateful to Dr. J. Ashley of the Office of Population Censuses and Surveys and Dr. N. Robinson of the London School of Hygiene and Tropical Medicine for allowing me access to original data for pelvic inflammatory disease.

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Frequency and distribution of salpingitis and pelvic inflammatory disease in short-stay hospitals in the United States

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Pelvic inflammatory disease is not a reportable condition in the United States. Based on data extracted from the Hospital Discharge Survey conducted by the National Center for Health Statistics, this article presents estimates of the frequency and distribution of PID in short-stay hospitals from 1970 through 1975. (AM. J. OBSTET. GYNECOL. 138:905, 1980.)

PELVIC INFLAMMATORY DISEASE is not a reportable condition in the United States. However, the National Center for Health Statistics (NCHS) conducts the Hospital Discharge Survey (HDS),[1] an ongoing sampling of short-stay hospital discharge records which provides essentially unbiased estimates of the frequency of any medical condition coded in the International Classification of Diseases.[2] The purpose of this article is to present estimates of the frequency and distribution of PID in short-stay hospitals in the United States as measured by the HDS.

Methods

The HDS consists of a stratified random sample of approximately 5% of the 7,900 civilian hospitals in the United States which have an average patient stay of less than 30 days. Within each of the sample hospitals, there is a further random sampling of hospital discharge records. Overall, the sample consists of over 200,000 hospital discharge records annually or about one of every 160 of the 35,000,000 patients discharged from short-stay hospitals annually in the United States.

Information from each sample discharge record is transcribed to a study abstract, which provides for patient demographic data and as many as five final discharge diagnoses and three operations. The principal diagnosis, when indicated, is listed first on the abstract. Otherwise, diagnoses are listed on the abstract in the order they are listed on the face sheet of hospital medical record. Diagnoses and operations for the years studied herein were coded according to the *Eighth Revision, International Classification of Diseases, Adapted for Use in the United States* (ICDA).

A patient weight, the reciprocal of the probability of sample selection, is recorded for each patient for the purpose of inflating the sample to provide estimates for the universe of short-stay hospital discharges.

We obtained HDS tapes from the NCHS for each of the years 1970 through 1975 for this study. We selected ICDA codes 612 (acute salpingitis), 613 (chronic salpingitis), 614 (unspecified salpingitis), and 616.0 (PID) to be the conditions of interest in this article. Diagnostic criteria could not be ascertained, but these diagnoses do represent the current and ordinary practice of medicine and nomenclature in United States hospitals.

As with any sample, there are probable errors associated with the derived estimates. In order to keep these sampling errors as low as possible, we aggregated the annual estimates and will present the data as mean annual numbers for the 6 year period 1970 through 1975. The probable errors of the estimates presented herein range from as low as ±2.3% to as high as ±30.0%, and depend on the magnitude of the estimate.

Because of the lack of uniformity of racial classification among the hospitals, racial designations were collapsed into the categories of "white" and "all others." Cases in which race was not specified were prorated to calculate population-based rates. For rates based on internally generated denominators, race was not prorated. For population-based rates, we used estimated

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Table I. Diagnosis of salpingitis and PID in short-stay hospitals, by reason for admission—annual average in United States, 1970-75

	Reason for admission			
Diagnosis	*Principal diagnosis (a)*	*% Total diagnoses (b)*	*Secondary diagnosis (c)*	*Total (d)*
Salpingitis				
Acute	10,510	81.1	2,451	12,961
Chronic	8,874	37.7	14,681	23,555
Unspecified	15,888	53.0	14,092	29,980
Total	35,272	53.0	31,224	66,496
PID	102,294	70.0	43,821	146,115
Grand total	137,566	64.7	75,045	212,611

Note: Column b calculated by (a ÷ d) × 100.

Table II. Median ages of women with diagnoses of salpingitis and PID in short-stay hospitals, United States, 1970-75

	Reason for admission	
Disease	*Principal*	*Secondary*
Salpingitis		
Acute	23	31
Chronic	29	38
Unspecified	26	33
Total salpingitis	26	35
PID	25	31
Grand total	25	33

Table III. Salpingitis and PID cases and rates per 100,000 female population over 14 years of age, by marital status—annual average in United States, 1970-75

Marital status	*Cases of salpingitis and PID*	*Cases/100,000 population*	
		Crude	*Age/race adjusted*
Married	114,194	248.8	286.9
Single	50,701	324.9	241.0
Widowed	6,074	63.2	257.8
Divorced	18,552	617.9	646.0
Separated	15,075	867.4	758.6
Total	204,596*	274.5	274.5

*Excludes unknown marital status and patients less than 15 years of age.

populations as of July 1, 1973, except for marital status rates. For the latter, the only population data available was that as of April 1, 1970.

Results

Table I summarizes the overall findings of the study. It lists the estimated annual number of diagnoses of salpingitis and PID in short-stay hospitals in the United States during the period 1970 through 1975. Salpingitis is divided into acute, chronic, and unspecified. PID is not similarly classified in the ICDA. In addition, the table divides the cases by diagnosis: those diagnoses constituting the principal reason for admission to the hospital and those diagnoses of salpingitis and PID which were secondary to some other principal cause for admission. Overall, there were 212,611 diagnoses of salpingitis and PID yearly, of which 146,115 were PID and 66,496 were salpingitis.

Chronic salpingitis (23,555 cases) was diagnosed about twice as frequently as acute salpingitis (12,961 cases) but almost half of the cases (29,980) were not specified as acute or chronic.

When the diagnosis was acute salpingitis, it was the principal cause for admission in 81% of all cases, but when the diagnosis was chronic salpingitis, it was the principal cause for admission in only 38%. Diagnosed salpingitis was the principal cause for admission in 53% of cases, compared to 70% for diagnoses of PID. Overall, salpingitis and PID were the principal reasons for hospital admission in 65% of cases.

The mean and median days of hospital stay for patients with salpingitis or PID as the principal diagnosis were 6.6 and 6.2 days, respectively. For salpingitis and PID as secondary diagnoses, the mean stay was 9.2 days with a median of 8.4 days.

Table II shows the median ages of hospitalized women with hospital diagnoses by stage of disease and by principal and secondary causes for admission. The median age of women with a principal diagnosis of acute salpingitis (23 years of age) is 6 years less than that of women with principal diagnoses of chronic salpingitis (29 years of age). In contrast, the median age of women reported with gonorrhea in the United States is 21 years. Women with a principal diagnosis of salpingitis and PID are consistently about 6 to 9 years younger than women with secondary diagnoses.

Racial differences in the rate of hospitalization for salpingitis and PID existed. The overall rate was 199 diagnoses per 100,000 population. For white women the rate was 154, and for all other races 509 per 100,000 population.

In Table III, we present hospital diagnoses of salpingitis and PID per 100,000 female population over 14 years of age by marital status for 204,596 of the 212,611 cases in which martial status was known. In the crude rate column, the highest risk groups are separated and divorced women (867.4 and 617.9, respectively). We computed age- and race-adjusted rates for the marital status groups to compensate for unequal age/race distributions within the marital status groups, and these adjusted rates are shown in Table III also. As with the crude rates, divorced and separated women are at greatest risk, but the rate for widowed women has increased to the extent that it parallels the risk for single and married women.

Table IV compares hospitalization rates for salpingitis and PID patients with those for gonorrhea patients for different regions of the United States. The first data column is the number of hospital diagnoses of salpingitis and PID per 1,000 women discharged, and the second column shows reported cases of gonorrhea in women per 1,000 population. The last two columns rank the salpingitis/PID and the gonorrhea rates from lowest to highest. The two sets of ranks either are identical or do not differ by more than one.

To examine the contribution of salpingitis and PID to total hospital admissions for women, we excluded admissions due to pregnancy (Table V). Thus, 10.3 of every 1,000 hospital admissions, excluding those for pregnancy, were necessitated by salpingitis and PID. For all ages and races, PID was the cause of hospitalization in one of every 100 women hospitalized in the United States during the years 1970 through 1975. The group at highest risk is 20 to 24 years of age. The risk in women over 40 years of age is quite low. The data column labeled "other" shows the admissions for salpingitis/PID among other than white women. The total admission rate of 31.5 for "other" is about four times higher than for white women. The highest rate in the "other" column is 90.6 diagnoses per 1,000 hospital admissions in the age group 20 to 24 years. The rate of 90.6 indicates that salpingitis and PID is the cause of one in every 11 hospital admissions among 20- to 24-year-old patients of races other than white, and that the rate among those 15 to 19 years of age is not much lower. These rates are based only on salpingitis or PID as the principal cause for hospital admission and do not include secondary diagnoses.

The frequency of hospital deaths among women with diagnoses of salpingitis/PID was low. The difference between mortality rates associated with principal and secondary diagnoses was not significant because of the very small number and high probable errors. The overall rate was 4.3 per 1,000 hospitalized women.

Table IV. Salpingitis (Salp.) and PID cases per 1,000 hospital discharges and gonorrhea cases per 1,000 female population in United States, by region

	Rates		*Rank*	
Region	*Salp./PID*	*Gonorrhea*	*Salp./PID*	*Gonorrhea*
New England	7.5	1.5	1	1
Mid-Atlantic	8.2	2.2	2	2
E. North Central	10.3	2.6	5	4
W. North Central	8.3	2.3	3	3
South Atlantic	11.7	4.6	7	8
E. South Central	15.6	4.6	9	9
W. South Central	12.9	4.0	8	7
Mountain	9.9	2.8	4	5
Pacific	10.5	3.3	6	6
Total	10.8	3.1		

Table V. Admissions for salpingitis or PID per 1,000 total women admitted excluding pregnancy admissions, by age and race—annual average in United States, 1970-75

Age (yr)	*White*	*Other*	*Unknown*	*Total*
15-19	22.3	85.4	29.5	31.5
20-24	30.2	90.6	35.9	40.2
25-29	22.6	61.0	29.4	28.8
30-34	17.2	42.2	14.9	20.2
35-39	9.9	24.2	13.1	12.2
40-49	5.1	10.2	4.5	5.5
50+	0.5	1.1	0.6	0.6
Total	7.7	31.5	9.9	10.3

Table VI summarizes the disposition of hospitalized patients with respect to surgical procedures. Surgery was performed on 42.3% of the patients with principal diagnoses of salpingitis or PID and 76.1% of women with secondary diagnoses. Among the 212,611 hospitalized women, slightly more than half (54.3%) underwent operations, and of these, 45.6%, or 52,634, were subjected to hysterectomies. Since multiple procedures were sometimes performed, the number of surgical procedures is greater than the number of patients who underwent operation.

Discussion

Salpingitis and PID are major health problems in the United States.[3-5] Excluding secondary diagnoses, one of every 560 women in the United States is hospitalized

Table VI. Number and types of surgical procedures on patients with diagnoses of salpingitis and PID—annual average in United States, 1970-75

	Reason for admission			
	No. with principal diagnosis (%)		*No. with secondary diagnosis (%)*	
Disposition				
No surgery performed	79,311	(57.7)	17,951	(23.9)
Surgery performed	58,256	(42.3)	57,094	(76.1)
Total	137,566	(100.0)	75,045	(100.0)
Type of surgical procedures				
On ovaries	28,830	(30.3)	31,096	(33.0)
On fallopian tubes	12,654	(13.3)	8,644	(9.2)
Hysterectomy	21,734	(22.8)	30,900	(32.8)
Other operations on uterus and supporting structures	12,560	(13.2)	11,738	(12.5)
Exploratory laparotomy or celiotomy	8,170	(8.6)	4,055	(4.3)
Other operations	11,204	(11.8)	7,729	(8.2)
Total	95,152	(100.0)	94,162	(100.0)

annually because of salpingitis or PID. These hospitalized women utilize 897,000 hospital days or 2,500 bed-years annually. This is the equivalent of six 400 bed hospitals just to care for patients with salpingitis or PID. Our data clearly indicate the adverse effect of the salpingitis/PID syndrome on fertility. Young women are at high risk and, if hospitalized, are quite likely to end up with permanent surgically induced infertility.

This article does not purport to determine the cause of PID, but the similarity of distribution of salpingitis and PID with that of sexually transmitted diseases emphasizes that sexually transmittable diseases probably play a major etiologic role.

Salpingitis and PID are used interchangeably in the United States. Although the conditions described by these two words are quite similarly distributed, there is one small difference. Although PID is not subclassified as acute or chronic, PID is more often the principal cause for hospital admission. Since acute disease is more likely than chronic to be the principal cause for admission, this suggests that the nomenclature "pelvic inflammatory disease" is more likely to be used for acute conditions and salpingitis more likely to be used for chronic conditions.

Starting with 1979 data, the International Classification of Diseases will subdivide PID into acute and chronic categories. When these 1979 data become available, we will be able to test the hypothesis that there may be some differences in the use of the words "salpingitis" and "PID."

Regardless of terminology, one fact remains clear: PID is a major health problem in the United States and long-term sequelae will become more evident as time passes.

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Pelvic inflammatory disease among women presenting to emergency rooms of hospitals in Massachusetts

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We obtained reports prospectively from the emergency rooms of 24 hospitals for 1 year. Nine percent of 3,505 women examined for genital infection with *Neisseria gonorrhoeae* were infected with this organism. Two thirds of the infected women had gonococcal pelvic inflammatory disease. The ratio of gonococcal PID to nongonococcal PID was 1:4.6. *N. gonorrhoeae* was isolated from 20% of women with PID in cities where the rate of all reported gonococcal infection was higher than the rate for the entire state (200 per 100,000 population). In contrast *N. gonorrhoeae* was isolated from only 13.4% of women with PID in cities with gonococcal infection rates lower than the rate for the state as a whole ($p < 0.01$). (AM. J. OBSTET. GYNECOL. 138:909, 1980.)

THE HOSPITAL emergency room plays an important role in contemporary health care delivery. Although its use is often a matter of convenience when private medical care is not available, the emergency room also can be expected to attract the more severely ill patients with any given disease. Infections due to *Neisseria gonorrhoeae* are no exception. Two studies,[1, 2] each conducted in a large urban hospital, found that infection with *Neisseria gonorrhoeae* in women was more likely to be detected in the emergency room than in other areas of the hospital, and that pelvic inflammatory disease was found frequently among infected women presenting to the emergency room. Rendtorff and associates[1] noted that over half of the reports of positive cultures for *N. gonorrhoeae* in Memphis came from one hospital and that many of the positive cultures from that hospital had been made in the emergency rooms. At Boston City Hospital, we found that the clinical spectrum of gonococcal infection in women varied from area to area in the hospital.[2] Infected women seen in the venereal disease clinic and in the obstetrics and gynecology clinic had few if any symptoms, whereas those seen in the emergency room were likely to have PID. Over 10% of the cultures made in the emergency room contained *N. gonorrhoeae* and many of the infected women had PID. Since both of these studies used a positive culture for *N. gonorrhoeae* as the starting point, no data were recorded for women with nongonococcal PID. Both of these studies were conducted in hospitals located in areas of high gonorrhea morbidity. The findings stimulated us to look at prevalence rates for PID in emergency rooms of hospitals in areas where gonorrhea morbidity was more representative of the population as a whole.

Methods

A prospective study was conducted from April, 1977, to March, 1978, to obtain information on women examined for gonococcal infection in emergency rooms of hospitals in the Commonwealth of Massachusetts. In the state a sample of 51 of the 204 hospitals with emergency rooms was selected initially on the basis of location within the six Health System Areas in Massachusetts in 1976. All 51 hospitals were visited in order to discuss the emergency room management of women with gonorrhea and with PID. A protocol was developed and reviewed with the individual responsible for collecting the data. Each hospital was asked to provide us with quarterly data on the results of endocervical cultures for *N. gonorrhoeae* and on PID. The diagnosis of PID was made by the examining physician and was based on clinical manifestations such as lower abdominal pain, lower abdominal tenderness, adnexal tenderness, and pain on manipulation of the uterus. We did not review the patient's medical records. If the

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Table I. PID and the isolation of *N. gonorrhoeae* from endocervical specimens from women presenting to emergency rooms in 24 hospitals in Massachusetts

		N. gonorrhoeae *isolated*		*PID*			
				Gonococcal			
Dates	*No. examined*	*No.*	*%*	*No.*	*%**	*No. nongonococcal*	*Ratio†*
April-June, 1977	882	82	9.3	57	70	209	1:3.7
July-September, 1977	1,049	94	9.0	53	56	256	1:4.8
October-December, 1977	923	81	8.8	52	64	267	1:5.1
January-March, 1978	651	60	9.2	39	65	204	1:5.2
Total	3,505	317	9.0	201	63	936	1:4.6

*Percent of women with positive cultures for *N. gonorrhoeae.*
†Ratio of gonococcal to nongonococcal PID.

Table II. Relation of all reported cases of gonorrhea to the prevalence of gonococcal infection among women with PID presenting to emergency rooms in 24 hospitals in Massachusetts

		N. gonorrhoeae *isolated*	
Reported cases of gonorrhea/ 100,000 population	*No. with PID*	*No.*	*%*
>200	703	143	20.3
≤200	434	58	13.4

hospital's laboratory reported that the endocervical culture from such a patient contained *N. gonorrhoeae,* she was considered to have gonococcal PID. A diagnosis of nongonococcal PID was assigned if the culture did not contain gonococci.

At the conclusion of the study, a questionnaire was mailed to each hospital to verify whether or not it used the criteria developed for this study. Twenty-four hospitals provided consistent data for the entire study period and are included in this report. These hospitals were representative of the original 51 selected in the six Health System Areas. At least three hospitals from each Health System Area were included.

Results

Table I summarizes the data reported by the 24 participating hospitals. About 9% of the endocervical cultures from women contained *N. gonorrhoeae.* Fewer women were examined during January to March of 1978 when severe storms crippled eastern Massachusetts and limited access to emergency rooms. Despite the decrease, the percentage of examined women from whom gonococci were recovered was similar to that seen during the previous quarters. The yield from testing in the emergency room was compared with the yield obtained in screening programs in Massachusetts during the same months when 1,337 (1.5%) of 89,733 cultures contained *N. gonorrhoeae.* Each Health System Area showed a higher yield of positive cultures in emergency rooms than in the screening program. On the other hand, the total number of women tested and found to have gonorrhea was lower in emergency rooms than in the screening program.

Of 317 women from whom gonococci were recovered in emergency rooms, 201 (63%) had PID. In contrast, PID was documented in only 108 (5.2%) of 2,044 women from whom *Neisseria gonorrhoeae* was isolated during the same 12 months in venereal disease clinics supported by the Massachusetts Department of Public Health.

The majority of women with PID had negative cultures for *N. gonorrhoeae.* This finding was true statewide. Differences in the ratio of gonococcal to nongonococcal PID were observed in cities of high and low rates of gonorrhea morbidity. As shown in Table II, women with PID were more likely to have a positive culture for *N. gonorrhoeae* if they presented to hospitals in areas with a higher than average rate of reported gonorrhea ($p < 0.01$).

Discussion

These data emphasize the importance of emergency rooms in the diagnosis and management of gonococcal infection. Nine percent of the women who had endocervical cultures for *N. gonorrhoeae* were infected, a yield per patient examined of almost an order of magnitude greater than that obtained in screening programs (1.5%). Although the technique of obtaining and handling endocervical culture specimens was not observed by us, the yield of 9% gonococcal isolation is comparable to that reported from Boston City Hospital, where 169 (12.3%) of 1,377 cultures obtained from women attending the emergency room contained gonococci.[2] Furthermore, two thirds of these infected women had PID, the most devastating complication of gonococcal infection. Only about 5% of women found to have gonococcal infection in venereal disease clinics had this important complication.

In the past, the view of the clinical spectrum of gonococcal infection has been based largely on studies conducted in venereal disease clinics, primarily because data from these facilities were readily available to those who conducted the studies. These studies have given rise to the widely accepted notion that gonococcal infection in women is primarily nonsymptomatic. As indicated by the data presented herein and in other recent studies,[1, 2] the hospital emergency room plays an important role in the diagnosis and treatment of gonococcal disease. This role has been poorly recognized because data are harder to collect in 24 hour emergency rooms than in clinics operating with less heroic schedules, and because the traditional public health recording system has not been adapted to receive data collected in areas other than venereal disease clinics. Consequently, all emergency rooms should have the facilities and the staffing to enable them to detect PID in women who present with abdominal symptoms and to detect *N. gonorrhoeae* in endocervical cultures from these patients.

As important as is gonococcal PID, only about 20% of patients with PID had evidence of gonococcal infection. This percentage was observed consistently in each quarter and in each geographic area. Clinical impressions of PID may be inaccurate. To judge the reliability of the gonococcal/nongonococcal PID ratio (1 : 4.6), we obtained data from patients at the Massachusetts General Hospital (not included in this study). In calendar year 1976, there were 19 patients with gonococcal and 68 with nongonococcal PID, a ratio of 1 : 3.6, which is similar to that noted in our study.

Not surprisingly, the likelihood that a woman presenting with PID would have gonococcal infection was proportional to the reported gonorrhea morbidity in her community. Indeed, the lowest gonococcal/nongonococcal ratios (1 : 1.8) were seen in two hospitals located in Boston and Springfield, cities which have the highest reported rates of gonococcal infection in the Commonwealth. It is likely that in areas where gonococcal infection is seen infrequently, such as in many private practices, most cases of PID (often euphemistically termed "endometritis") will be nongonococcal.

Several important considerations emerge from these data. First, since treatment for PID must be initiated when the patient is first seen and since gonococcal and nongonococcal disease cannot be reliably differentiated clinically,[3, 4] treatment schedules should be adequate for both the gonococcal and nongonococcal forms. Currently recommended treatment schedules[5] have been chosen primarily because of their efficacy in gonococcal PID. Since nongonococcal disease is less responsive to initial treatment[4] and is more likely to result in long-term complications[4, 6] compared to gonococcal infection, adequate treatment is important. Studies assessing the short- and long-term efficacy of various treatment schedules for PID, gonococcal and nongonococcal, are urgently needed.

Treatment of PID does not end with making a clinical diagnosis and prescribing an antibiotic. Only about 20% of patients with PID are hospitalized where they can be carefully monitored. All too often, the majority who do not require hospitalization are given a prescription for an oral antibiotic and told to make an appointment to see their gynecologist in a week or two. Closer observation of these patients is warranted. One approach is to give the patient an appointment to be seen in 2 or 3 days. This visit can occur in a private office, a hospital gynecology clinic, or in a venereal disease clinic. During this visit, the results of the pretreatment cultures can be discussed with the patient. If *N. gonorrhoeae* was recovered from the pretreatment cultures, treatment of male sexual partners, who are often nonsymptomatic,[4, 7] can be undertaken while the index patient is still being treated. In addition, the patient can be reexamined to ensure that the initial diagnosis of PID was correct and that the patient is responding satisfactorily to the initially prescribed treatment. If not, hospitalization or a change of antibiotics may be required.

The management of the sexual partners of women who have nongonococcal PID is not well defined. Some of these men will be infected with *C. trachomatis*. Others will not be infected with any agent for which treatment is currently indicated. Since cultures for *C. trachomatis* are not available to most clinicians, an approach must be developed that does not depend on the results of chlamydial cultures. One such approach would be to examine male sexual partners of women who have nongonococcal PID for pyuria. Those who have pyuria could be treated for presumed chlamydial urethritis. Another approach would be to administer tetracycline to all sexual partners of women with nongonococcal PID. This important area requires further study.

We acknowledge the assistance of John Narkunas, John Sirmon, Barbara Carlson, Roger Kelly, Marion Malinowski, Estelle McGonough, Elizabeth Penhy, Therese Rheaume, Helen Urgilevitch, and Marie Ceraso.

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Pelvic inflammatory disease and its consequences in the developing world

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THE PATTERNS OF pelvic inflammatory disease, its consequences and its underlying causes, its impact on health and health services, and the alternative strategies for control vary as widely between developing countries as between developed and developing countries. In the developed countries, concern over the problem of PID had been focused on the role of sexually transmitted disease (STD), the role of different microorganisms in the etiology of acute salpingitis and their consequences, diagnostic procedures and optimal therapy, and public health strategies for the control of STDs. As a result of the clinical and epidemiologic studies of research groups in Sweden and the United States, a model of the problem of PID and its consequences has been developed and may be used both in understanding the disease and in evolving a strategy for control. A simplified representation of that model is shown in Fig. 1. In the developed world, the incidence and epidemiology of STD infections and the patterns of contraceptive use are among the important factors affecting PID levels and its sequelae, particularly infertility and ectopic pregnancy.

Understanding the epidemiology of PID and its consequences in the developing countries is not nearly as simple. Aside from the very wide variations among developing countries in the prevalence of *Neisseria gonorrhoeae* and the other STD organisms, such factors as access to and use of health services, differential risks of puerperal sepsis and of infection after abortion, the prevalence of genital tract tuberculosis, and the prevalence of such endemic diseases as schistosomiasis and filariasis may affect the patterns of PID and its consequences. The model of PID that may be more applicable to the developing world is presented in Fig. 2.

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As in the case of the developed countries, it may be possible to use the model as a guide to epidemiologic research and to developing a strategy for the control and prevention of PID and its consequences. It may thus be possible to estimate the relative importance of STD, the effects of infection post partum or post abortion, and the contribution to the problem of other endemic diseases such as tuberculosis, schistosomiasis, and filariasis.

Prevalence of pelvic inflammatory disease

There are little data from developing countries on the incidence of PID per se, but despite this, there are indications that it is a problem of some magnitude. Lithgow and Rubin[1] state that pelvic sepsis is probably the commonest gynecologic disease afflicting the African woman; cases of pelvic sepsis comprise approximately one third of admissions to any large Southern African Bantu gynecology unit. Griffiths[2] states that in certain tribes in Uganda, up to 50% of the women are infertile as a result of the tubal occlusive sequelae of gonococcal PID. A similar finding was made by Scragg[3] in investigating the depopulation of several areas of New Ireland. In certain tribes, 74% of the married couples were infertile, and on hysterosalpingography, 80% of the female partners showed degrees of tubal occlusion sufficient to suggest infertility.

As an index of the frequency of the problem, some authors site the percentage of gynecologic admissions owing to PID. Since the universe from which the cases are drawn is seldom defined, and the number of cases included depends largely on the degree of awareness on the part of personnel and diagnostic criteria, there is little comparability between data and no wider inferences can be drawn of the overall prevalence other than to note that, for many countries, PID constitutes a serious health problem (Tables IA and IB).

Many authors focus only on acute salpingitis, excluding chronic PID and infections post partum and post abortion. As noted in Tables II and III, the proportion

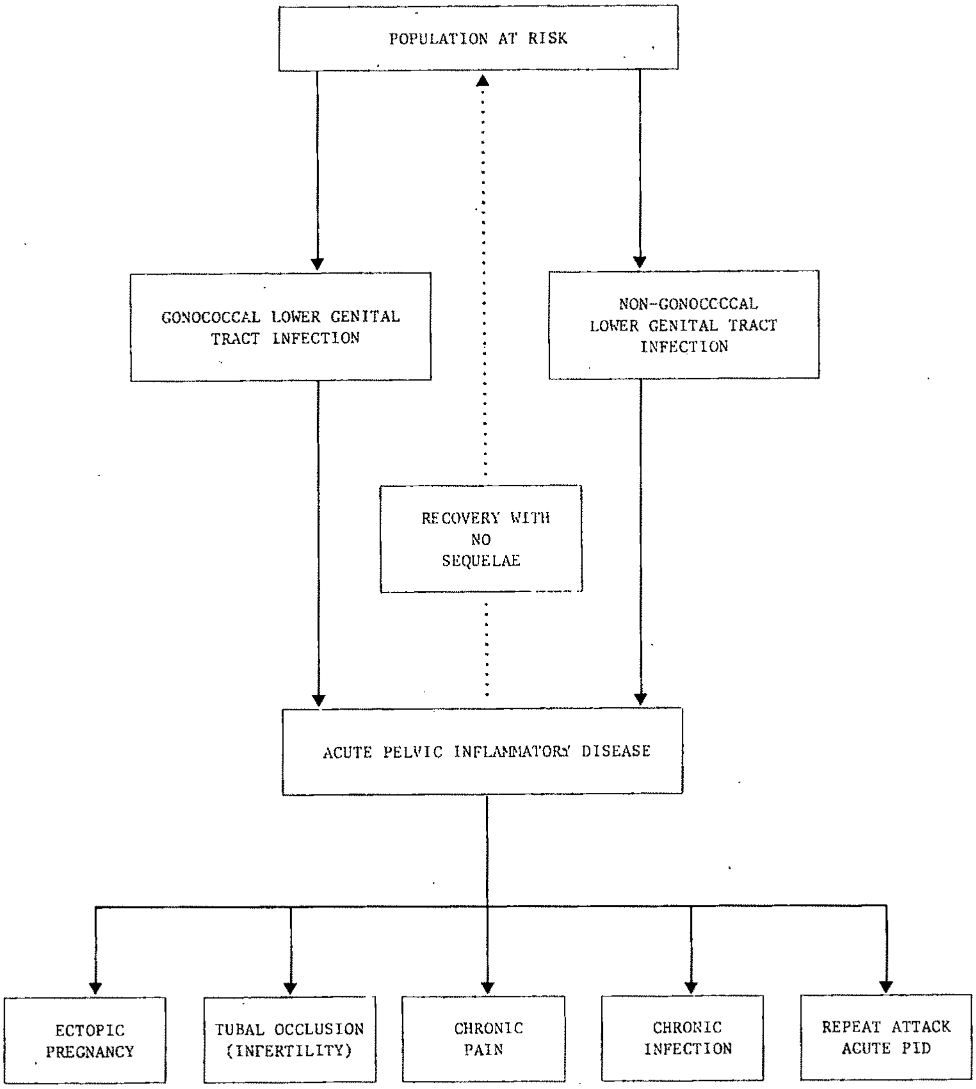
POPULATION AT RISK
GONOCOCCAL LOWER GENITAL TRACT INFECTION
NON-GONOCOCCAL LOWER GENITAL TRACT INFECTION
RECOVERY WITH NO SEQUELAE
ACUTE PELVIC INFLAMMATORY DISEASE
ECTOPIC PREGNANCY
TUBAL OCCLUSION (INFERTILITY)
CHRONIC PAIN
CHRONIC INFECTION
REPEAT ATTACK ACUTE PID

Fig. 1. A simplified model of PID in developed countries.

of acute to chronic cases of PID may vary widely and provide a clue as to the relative role of STD, infections post partum and post abortion, and tuberculosis in the overall problem of PID.

In the African setting, acute salpingitis, chronic salpingitis, and puerperal sepsis are all commonly encountered. In the Indian experience, the number of cases of acute salpingitis was small, as was the rate of recovery of *N. gonorrhoeae*, whereas chronic PID was most common among all cases. In another Indian center, 26.4% of infertility cases were associated with tubal pathology, with nearly 40% of these cases being tuberculous in origin.[4, 5] It appears that tuberculosis, puerperal sepsis, and infections post abortion are the most important underlying causes of PID in the Indian setting.

Age structure

A further differentiation of PID related to the STDs and PID related to obstetric events might be obtained by examining the age-specific rates of either acute PID or salpingitis and the rates for chronic PID. The curve of the age distribution for patients with acute PID in Uganda[6] (Fig. 3) bears a close resemblance to the age-specific rates seen by Wright and Laemmle[7] in an indigent American population (Fig. 4).

It remains to be established whether or not the age distribution of patients with chronic PID differs from that of patients with acute disease.

A correlation between age of sexual debut and an increased risk of PID has been suggested. Grech and associates[6] found that in Kampala, Uganda, 83.8% of patients with PID had their sexual debuts before the

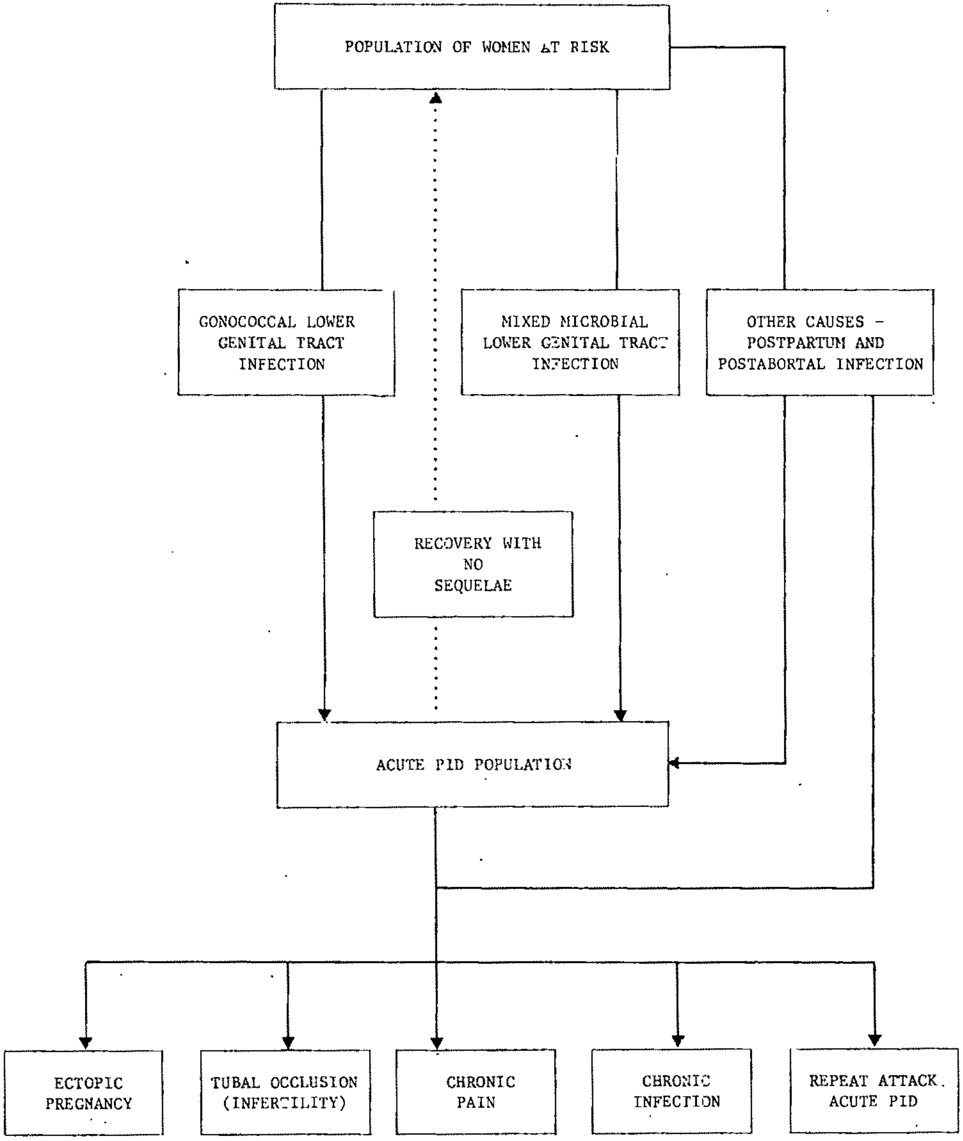
POPULATION OF WOMEN AT RISK
GONOCOCCAL LOWER GENITAL TRACT INFECTION
MIXED MICROBIAL LOWER GENITAL TRACT INFECTION
OTHER CAUSES - POSTPARTUM AND POSTABORTAL INFECTION
RECOVERY WITH NO SEQUELAE
ACUTE PID POPULATION
ECTOPIC PREGNANCY
TUBAL OCCLUSION (INFERTILITY)
CHRONIC PAIN
CHRONIC INFECTION
REPEAT ATTACK. ACUTE PID

Fig. 2. An etiologic model of acute PID and its sequelae in developing countries.

age of 16 years, although no control figures are provided. This should be viewed in the light of the finding by Weström and colleagues[8-11] who estimated a relative risk of salpingitis of 1:8 for sexually active 15-year-old girls as opposed to a risk of 1:80 for 24-year-old women.

A similar association between age at onset of sexual activity and prevalence of infertility was noted in the Cameroon, by Falahala[12] (Table IV).

Prevalence/incidence of sexually transmitted diseases

There are two sources of data on either the incidence rate or the prevalence of gonorrhea and other STDs: national statistics of reported cases and ad hoc population surveys. National statistics for gonorrhea represent minimal estimates because of variations owing to underreporting. National incidence rates of varying reliability for some developing countries are presented in Table V.

Surveys of defined population groups using standardized diagnostic and laboratory procedures represent a more reliable estimate of a limited population. Prevalence or incidence data from studies in several African countries are presented in Table VI. The prevalence rates of *N. gonorrhoeae* among asymptomatic individuals are no lower than 1%, except in one district in Zaire, and they are as high as 14% among asymptomatic pregnant women in Cameroon.

Between countries and between population sub-

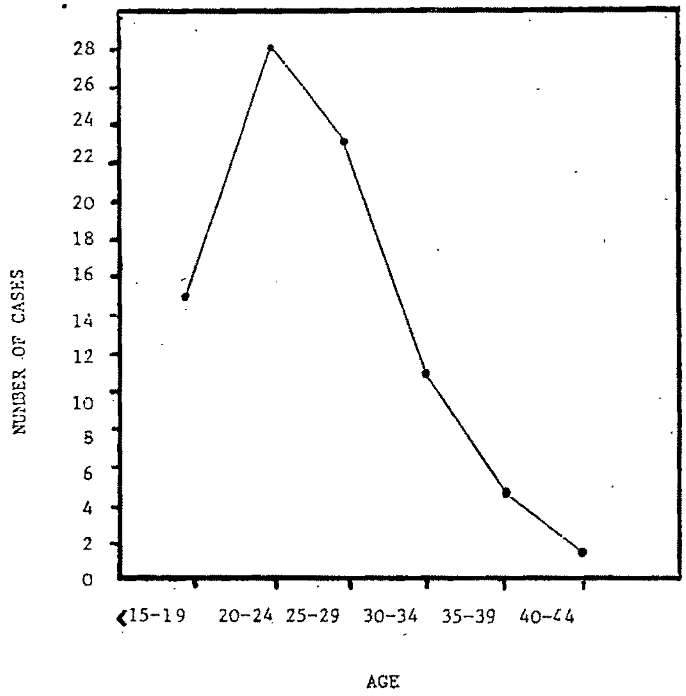

Fig. 3. Age-specific case rate, Uganda. (From Grech and associates.[6])

Table IA. Percent admissions to gynecology services for PID—published reports from Africa

Author	*Country*	*% Admissions for PID*
Weissenberger et al.[46]	Zimbabwe	44
Grech et al.[6, 47]	Uganda (1972)	30.7
Silbert[48]	South Africa (Cape, colored consultations)	25
Trussel[23]	Uganda (1968)	21.95
Lithgow and Rubin[1]	South Africa (Bantu)	15.8

groups within a country there are wide variations in the prevalence rates for gonococcal infection. In Zimbabwe, family planning clinic attendees had a 12% prevalence rate of *N. gonorrhoeae* compared to a rate of 2% for antenatal clinic attendees (Table VI). For the same categories of women in the Cameroon, the rates were reversed, 1.9% rate of *N. gonorrhoeae* among asymptomatic attendees of family planning clinics and 13.6% among asymptomatic pregnant women. Even within a family planning clinic population, rates vary, for example, with such factors as marital status in Kenya. In one area of Uganda between 8% and 10% of *all* health clinic consultations were for gonococcal infections.

The role of pregnancy as a factor increasing the risk of gonococcal infection cannot always be ascertained by comparing the prevalence rates for gonorrhea among antenatal patients and control groups because of possible selective bias and differing sexual patterns both by social grouping and by pregnancy status. As previously noted, the prevalence rates of gonorrhea among pregnant women and attendees of family planning clinics in a Zimbabwe study are the inverse of those in a study from the Cameroon. However, in a field study in a rural area of Uganda where the risk of exposure to gonococci was the same for pregnant and nonpregnant women, Arya[13] noted a significantly higher rate of gonococcal isolations in pregnant women (40%) as compared with nonpregnant women (19.8%). This observation, coupled with that of Nasah and Evang[14] of similar rates of gonorrhea in pregnant women regardless of presence or absence of symptoms (Table VI), suggests an important role for gonococci and other STDs in the etiology of puerperal sepsis or infection post abortion, particularly as they note that the isolation of gonococci in cases of puerperal sepsis was twice that from nonseptic patients post partum.

Incidence rate of PID in a population of women with lower genital tract infections

The rate at which PID develops in the subpopulation with gonorrhea has been estimated to be 10.8% in Sweden.[8-11, 15] However, no data are available indicating at what rate chlamydial or other microbial infections of the lower genital tract progress to PID. The only data available are on the proportion of cases of

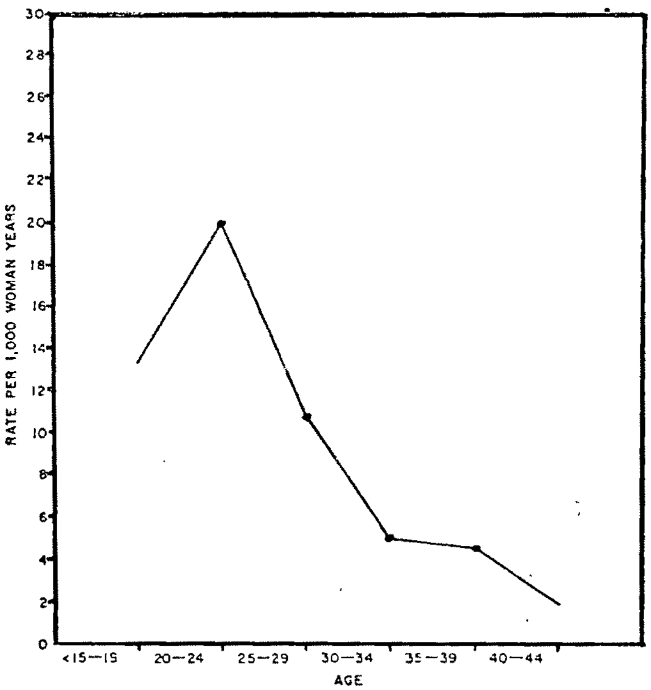

Fig. 4. Age-specific rates for acute first "definite" episodes of PID, 1965. (From Wright and Laemmle.[7])

Table IB. Distribution of gynecologic admissions by diagnosis per year—WHO Collaborating Centres for Clinical Research in Human Reproduction

Hospital	*No. gynecologic admissions*	*Admissions for PID*		*Admissions for puerperal sepsis*		*Admissions for septic abortion*		*Admissions for ectopic pregnancy*	
		No.	*% Total*	*No.*	*% Total*	*No.*	*% Total*	*No.*	*% Total*
India									
1	2,956	250	9.36	22	0.85	19	0.60	30	1.00
2	1,956	30	1.50	17	0.90	20	1.02	20	1.02
3	4,115	24	0.58	69	1.70	49	1.19	12	0.30
Pakistan	6,787	19	0.30	293	4.32	95	1.40	36	0.53
Philippines	1,328	30	2.3	38	2.90	132	9.94	120	9.04
Thailand									
1	1,638	135	8.19	88	5.34	164	9.95	90	5.46
2	2,070	60	2.90	42	2 03	658	31.79	133	6.42
Zambia	5,355	664	12.40	158	2 95	91	1.70	75	1.40
Tanzania*	6,636	757	11.40	NA†	—	NA	—	134	2.08
Egypt	4,619	62	1.34	195	4.22	86	1.86	36	0.78
Malaysia*	2,736	7	0.26	2	0.07	63	2.3	54	1.97
Australia	9,177	115	1.25	8	0.08	6	0.06	82	0.89
Hungary	889	150	16.87	12	1.35	7	0.79	25	2.84
Yugoslavia	8,418	873	10.37	40	0.48	4	0.05	139	1.65
Cuba	4,163	138	3.31	91	2.19	11	0.26	37	0.89

*Not designated WHO Collaborating Centres for Clinical Research in Human Reproduction.
†Not applicable.

PID in which *N. gonorrhoeae* and other organisms are implicated. These proportions vary both temporally and geographically (Table VII). The reliability of the figures obviously is dependent on the adequacy of microbiologic diagnostic procedures. Other factors affecting the rate of gonococcus isolation in cases of PID include availability of and self-treatment with antibiotics, a common phenomenon in the developing world, and the evolution of antibiotic-resistant microbial strains.[16]

Chlamydia trachomatis. It has been suggested that *Chlamydia trachomatis* is the most common causative agent of STD in many developed countries.[17-19] It has been implicated as an etiologic agent in PID as well as

Table II. Description of 800 cases of PID diagnosed during 7 years in an Indian hospital*

	No.	*%*
Total cases	800	100.0
PID		
Acute	28	3.5
Chronic	772	96.5
N. gonorrhoeae		
History suggestive of infection	33	4.1
Isolated	11	1.4
Tuberculous	48	6.0
Postpartum	122	15.3
Postabortion	76	9.5
Postinstrumentation	75	9.3

*Data from Shah and associates.[21]

Table III. Composition of PID and associated cases in two African hospitals

	*South Africa**		*Uganda*†	
	No.	*%*	*No.*	*%*
Acute PID	334	60	37	16
Puerperal sepsis	85	15.3		
Chronic infection	26	4.7		
Pyosalpinx	72	12.9	34	14.7
Hydrosalpinx			19	8.2
Abscess	36	6.5	48	20.8
Tubercular PID	3	.5	2	0.9
Septic spontaneous symphysiotomy	1	.2		
Pyometria			1	0.4
Pelvic sepsis unspecified			93	40.3
Total	557		234	
% Gynecologic admissions		15.8		22

*Data from Lithgow and Rubin.[1]
†Data from Trussel.[23]

Table IV. Number of live births among married women more than 25 years of age by duration of premarital sexual activity, East Province, Cameroon*

Duration of premarital sexual activity (yr)	*Live births*	
	No. none (%)	*No. ≥1 (%)*
0	8 (6.0)	126 (94.0)
Up to 3	3 (9.4)	29 (90.6)
3-5	5 (17.9)	23 (82.1)
≥6	18 (24.0)	57 (76.0)

*Data from Falahala.[12]

Table V. Incidence rate of gonorrhea infection in developing countries, by year*

Country	*Yr*	*Incidence/100 adults/yr*
India	1977	0.15
Mexico	1977	0.43
Bolivia	1976	0.46
Argentina	1976	0.66
Upper Volta	1977	0.92
Uganda	1977	1.56
Kenya	1976	2.27
Colombia	1976	2.17
Mali	1977	2.91
Mauritania	1976	4.84
Bahamas	1976	6.67
Central African Republic	1977	7.51
Lesotho	1977	11.43
Gambia	1976	17.78
Greenland	1977	216.95

*Data from World Health Organization.[49]

lower genital tract infections, and in one series it was isolated in 35% of cases of confirmed salpingitis,[19] although no firm incidence rates are available. Background infection rates of 1% to 2% in asymptomatic men and 5% in asymptomatic women have been found in developed countries. The higher incidence in women is explained by the more rapid symptomatic response in men.

Punnonen and colleagues[20] have implicated *C. trachomatis* in infertility, showing that, matched against pregnant women, infertile women had a rate of antibody response to *C. trachomatis* of 57% (as opposed to 29%) a difference significant at $P < 0.001$.

The only data on chlamydial infection of the genital tract in Africa are the preliminary findings in the Cameroon by Professor P. C. Mafiamba* of serologic evidence of such infections in 50% of the population surveyed and serologic evidence of recent infection in 16%. At present, the numbers (63 serum samples) are insufficient to draw any conclusions regarding the association of infertility and chlamydiae.

Tuberculosis. Tubercular salpingitis, once accounting for a significant proportion of salpingitis cases throughout the world, is now a rarity in most developed countries. In many developing countries, tuberculosis remains a major health problem and genital tuberculosis accounts for a significant proportion of women presenting with infertility. Among 800 women with PID in one Indian series, 6% of cases were attributed to tuberculosis.[21] In another study of 125 patients with chronic PID, which included a laparoscopic investigation, 59 (49%) were found to have tuberculous genital tract infection,[5] while nearly 13% of 491 cases of primary infertility were attributed to pelvic tuberculosis.[4] In Ibadan, Ojo and co-workers[22] found that 3.5% of patients presenting with infertility had genital tuberculosis.

Infections post partum and post abortion. In many developing countries, delivery of infants most often takes place in the home, under the care of traditional

*Unpublished data.

Table VI. Prevalence and incidence rates for *N. gonorrhoeae* in African countries, by type of study

Country	*Author*	*Type of study*	*No. individuals in sample*	*Prevalence or incidence*
Uganda	Arya et al.[50]	Prevalence in samples		
		Teso (rural)	613 (343 women)	7.5%
		Ankole (rural)	—	1.20%
		Kampala	—	15.0%
		Kasangati (semirural)	—	2.76%
	Arya and Lawson[51]	Incidence rate in male university students	1,200 at risk	28.5%/yr for STD 18.8% GC*/yr
	Bennett[52]	Attendees at a rural health service	27,620	8%-10% of *all* health clinic attendances
Kenya	Hopcraft et al.[53]	Prevalence		
		Married users	50	12.0%
		Married nonusers	50	5.0%
		Unmarried users	50	28.0%
		Unmarried nonusers	50	24.0%
	Verhagen[54]	Incidence based on attendees/population	—	2.0%
Zaire	Romaniuk[55]	Prevalence among women surveyed in 1957 in five districts	—	3.8% 2.7% 2.5% 1.6% 0.4%
Cameroon	Nasah and Eyang[14]	Prevalence		
		Pregnant women		
		Symptomatic	610	15.2%
		Asymptomatic	110	13.6%
		Family planning clinic		
		Symptomatic	31	21.0%
		Asymptomatic	53	1.9%
		Immediate post-partum		
		Symptomatic	42	21.4%
		Asymptomatic	296	10.2%
Rhodesia (Zimbabwe)	Weissenberger et al.[46]	Prevalence among subjects in		
		Gynecology clinic	118	11.0%
		Antenatal clinic	50	2.0%
		Family planning clinic	100	12.0%
Nigeria	Osoba et al.[56,57]	Prevalence among		
		Asymptomatic pregnant women	208	3.4%
		Symptomatic women	228	17.0%
		Asymptomatic women	130	5.0%
		Infertile women	151	1.3%

*Gonococci.

Table VII. Relationship between occurrence of gonococcal infection and acute PID, by country

Author	*Country*	*% Incidence of PID in women infected with GC**	*% PID cases associated with presence of gonococcal infections*
Falk[17]	Sweden	10-15	41
Weström and Mårdh[9]	Sweden	20	50 (1965)
		10.8	9.7 (1973)
Jacobson and Weström[58]	Sweden	—	36
Eschenbach et al.[26]	U.S.A.	—	44
McBrown and Cruikshank[59]	Zimbabwe	—	4
Nasah and Eyang[14]	Cameroon	—	12
Grech et al.[6]	Uganda	—	36
Carty et al.[60]	Kenya	—	43
Shah et al.[21]	India	—	1.1 Proven 4.1 Presumed

*Gonococci.

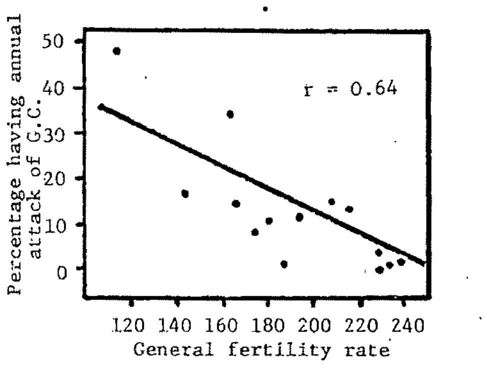

Fig. 5. General fertility rate and gonococcal infection rates in 14 Ugandan districts. (From Griffiths.[2])

midwives. In addition, in some countries illegal abortion rates tend to be very high. In these circumstances, both postpartum and postabortion sepsis remain important as sources of maternal death and morbidity, and may be significant factors in the production of both acute and chronic PID (Tables II and III).

Several studies show that infections post partum and post abortion maintain their importance in the developing countries. Trussel[23] stated that the commonest conditions predisposing to PID in Uganda were gonorrhea and puerperal fever; this was confirmed by Grech, Everett, and Mukasa[6] who showed that 19.7% of PID cases followed either delivery or abortion.

The relative proportion of admissions associated with puerperal sepsis or septic abortion is noted in Table IB for hospitals in 11 developing countries.

The influence of other infectious agents. It is suggested that there are other infectious agents which occur in developing countries and which play a role in increasing the incidence of PID and its sequelae. In particular, schistosomiasis and filariasis have been suggested as etiologic agents.[24, 25] These infections might directly affect the fallopian tubes or might predispose to chronic local infections in association with the transient asymptomatic presence of microorganisms in the fallopian tubes.[26]

Schistosomiasis. Both *Schistosoma mansoni* and *S. haematobium* may be found in the genital tract, but *S. haematobium* is the more common in the pelvic organs.[27] In a study from Zimbabwe, Gelfand and associates[28] established an incidence of genital involvement of 50% in autopsies of 64 consecutive women. The lesions involved mainly the deposition of ova in tubal tissues. Mouktar[29] cites case studies in Egypt indicating the occurrence of schistosomal salpingitis, both chronic and acute. In Malawi, Bullough[27] reported a significantly higher rate of *S. haematobium* infections in patients with primary and secondary infertility (41.8% and 38.0%) as compared to fertile control women admitted for incomplete abortion (21.4%).

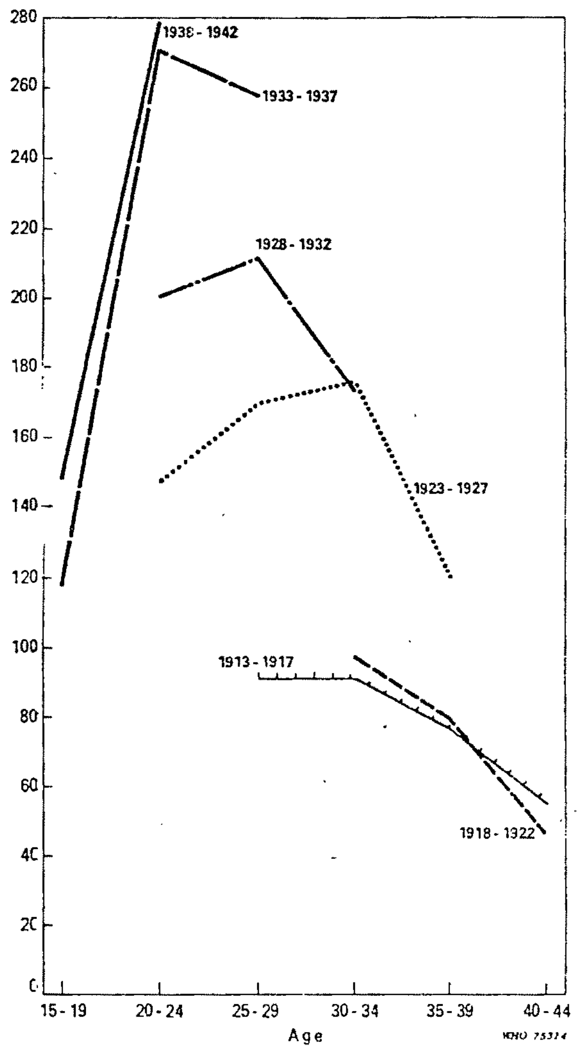

Fig. 6. Age-specific birth rates by 5 year cohorts of women born between 1913 and 1942 in New Ireland. (Adapted from Ring and Scragg.[36])

The significance of schistosomal involvement may lie in its weakening and irritation of tubal tissues, which renders them more vulnerable to secondary infections. There is also a suggestion that ectopic pregnancy rates are higher in women with bilharzia.[25]

Filariasis. Filarial infection involves the genital lymphatic system of both men and women. Romiti[30] suggests that involvement of broad ligament lymphatic drainage so weakens tubal tissue as to render it liable to secondary infection, and cites this as a mechanism of production of PID and tubal occlusion.

Modawi[31] ascribes areas of high infertility to filarial infection in the Sudan. Nasah and Cox[32] have suggested that filarial involvement of the testis is correlated with a local immune reaction in the testis and with the extent of oligospermia or azospermia in infertile men. Whether significant filarial lesions exist or are

Table VIII. Indices of fertility in various countries*

	% Never pregnant	*% Nulliparous*	*Secondary infertility*	*No. children born*	*Only one child born*	*Childless*
Nigeria						
Women married 2+ yr				1.6		
Women married 4+ yr					1.8	
Women married 2+ yr, aged 20-29 yr	3.4		11.1			
Woman married 2+ yr, aged 30-39 yr	3.6		15.5			
Zambia						
Women aged 35-39 yr, 8 provinces						
Central		13.0				
Copperbelt		14.0				
Eastern		11.0				
Luapula		13.0				
Northern		6.0				
Northwestern		24.0				
Southern		15.0				
Western		20.0				
Colombia						
Married women aged 35-39 yr						
Urban		13.0				
Rural		7.0				
Korea						
Married women aged 35-39 yr						
Seoul	1.0	1.0				
Other urban	2.0	3.0				
Rural	1.0	1.0				
Thailand						
Ever-married women aged 35-39 yr	1.5					
Women married 10-19 yr				1.5	5.1	
Pakistan						
Ever-married women aged 35-39 yr	4.0					
Women married 10-19 yr				4.0	4.0	
Papua New Guinea						
Women aged 35-39 yr						
Buka		2.0		2.0	6.4	
New Ireland		22.8		22.8	9.6	
Malaysia						
Currently married women aged 35-39 yr		1.9				
Women currently married 10-14 yr				2.7	3.5	
Women currently married 15-19 yr				0.7	2.5	

*Data from Belsey.[61]

associated with infertility in the woman still remains to be established.

Sequelae of pelvic inflammatory disease

Infertility. Many reports attempting to define the role of gonorrhea in infertility rely on data gathered from the male population; nevertheless, such reports as that of Griffiths[2] exemplify the correlation between such indicators as urethral stricture, *N. gonorrhoeae* attack rates, and overall fertility levels (Fig. 5).

In the absence of specific epidemiologic studies on the prevalence of infertility, estimates of the magnitude of the problem are often imprecise, being based either on demographic data or on health service statistics.

Several indices have been used to describe the magnitude and the distribution of infertility.[33] Rates of childlessness, nulliparity, and nulligravidity provide estimates of primary infertility. Furthermore, if one assumes that couples are not practicing voluntary infertility, i.e., the use of either traditional or modern methods of contraception, and that sexual intercourse is occurring regularly, then the failure to have a second child after a defined period of time, for example 10 to 14 years, could be used as an indirect indicator of secondary infertility.

The indices of primary infertility vary widely, being as low as 1.0% and 1.5% (nulligravid) for married women 35 to 39 years of age in Korea and Thailand, and as high as 13% and 23% for similar women in urban areas of Colombia and one rural area of Papua New Guinea (Table VIII), and even higher in some parts of Africa (Table IX). The higher levels of either childlessness or nulliparity in the urban areas of some developing countries, as represented by Colombia (Table VIII) suggest either that voluntary infertility rates are higher in the urban areas or that certain of the "ac-

Table IX. Indices of infertility* and general fertility in Central and West Africa

Country and/or region	*Population in 1,000s*	*General fertility rate (no. births/1,000 women aged 15-44 yr)*	*Crude live birth rate/1,000 population*	*% Childless women aged 25-29 yr*	*% Childless women aged 50+ yr*
Camaroon					
West	1,025	196	49.8	7.0	6.7
Southeast	1,135		36.4	28	23
North	1,395		41.0	21	15
Central African Empire	1,021	157	48	25.2	13.6
Banda	318	122	40.6		
Nzakara	30	48	20.0		
Baya	294	194	54.6		
Central region	240	125	41.0	34.7	15.0
West region	643	187	53	19.4	10.4
River region	134	101	36	36.3	19.0
Gabon	440	116	35	34	31.9
Wolen N'tem	78	122	37		31.2
Ogoone Lolo	37	80	25		46.2
Nyanga	37	170	52		17.8
Upper Volta	4,440	194	49.6	7.2	6
Niger	2,600	232	50.55	12.8	5.2
Mali (Toureg)	76	209	52	26	15
Senegal	3,049	178	43.3	12	5.6
Sudan	10,262		51.7		9.6
Bahr el Ghaza	991		84.6		4.2
Blue Nile	2,070		45.7		8.4
Darfur	1,329		41.8		7.3
Equatoria	904		54.1		21.2
Kassala	941		42.6		13.5
Khartoum	505		40.7		9.7
Kordofan	1,762		50.0		9.9
Northern	873		43.0		7.8
Upper Nile	889		69.3		2.3
Zaire	21,800	171	42.7	22.1	17.6
Kwango	466	203	48.1	6.8	3.4
S. Kivu	831	211	52.3	7.1	4.6
Equateur	302	133	33.7	39.1	40.0
Tahuapa	395	113	30.5	44.1	33.0
Stanleyville	635	123	34.0	34.4	23.3
Bea-Vele	468	64	19.1	50.7	37.3
Haut-Vele	589	83	23.2	46.2	36.9
Nanie-Ma	447	129	34.3	27.9	23.5
Congo†		145	41.1	17	15

*Data from Belsey.[33]

†Excludes Brazzaville and Pointe Noire.

quired" causes of infertility, such as PID, are more common in these areas.

The presumed role of gonococcal infection in infertility in Papua New Guinea is supported by the rise in the age-specific fertility rates in women whose reproductive careers benefited from a mass penicillin therapy program[3, 34] (Fig. 6).

An examination of the proportion of couples suffering from primary vs. secondary infertility may in some circumstances provide some suggestions as to the etiology of infertility in that particular setting. The variation in the rates of primary and secondary infertility reflects differences in the pattern of underlying causes. Clinic-based data generally suggest a predominance of primary infertility, which affects from 53% to 75% of couples seeking assistance.[35] This predominance of primary infertility may not hold true for many African clinics. For example, in one report from Mali, nearly two thirds of the 248 clinic consultations in a 15 month period involved couples suffering from secondary infertility.[36] Similar patterns have been reported from Gabon.[37] In contrast to that derived from clinical data, the impression from the various demographic indices of several developing countries is that secondary infertility occurs as frequently if not more frequently than primary infertility. For example, among women currently married 15 to 19 years in Malaysia, 0.7% have never borne a child, whereas 2.5% have borne only one child.

In the extreme situations where the levels of infertility are high and predominantly either primary or secondary, it may be possible to hypothesize a single underlying mechanism or cause. For example (Fig. 7), although high levels of infertility are noted in two districts in the southern Sudan, in one district the pattern is almost exclusively one of secondary infertility which suggests a major role for postpartum factors such as sepsis. In the other districts, the problem appears to be predominantly one of primary infertility, suggesting etiologic factors operating before or at the time of puberty which affect either ovulation or the patency of the fallopian tube or spermatogenesis.

In a series of 1,457 cases of laparoscopically confirmed PID studied by Weström and Mårdh,[9] there was an overall reduction in fertility of 21.2% in the post-PID population. There was an increasing risk of infertility with increasing numbers of attacks, with the risks of tubal occlusion after one, two, and three or more attacks of PID being 12.8%, 35.5%, and 75%, respectively.

Reports on hysterosalpingography and laparoscopy investigations of infertile women in the developing countries show that tubal occlusive phenomena are the predominant cause of both primary and secondary infertility, but the percent attributable to tubal occlusion varies widely (Table X).

Ectopic pregnancy. As a sequela of PID, the rate of ectopic pregnancy relative to normal delivery is increasing in many developed countries. Thus, ectopic pregnancy has attracted renewed attention, particularly since there appears to be an interaction among increasing rates of usage of the intrauterine contraceptive device (IUCD), PID, and ectopic pregnancy.[10, 11, 38]

The role of previous pregnancy or induced abortion on the occurrence of ectopic pregnancy can be estimated from data derived from the Stockholm computer register of hospital admissions (Table XI). From 1972 to 1975, the rate of ectopic pregnancy following a previous live birth was 1.2%, or an ectopic pregnancy/delivery ratio of 1 : 68. Comparable rates and ratios following an induced abortion are 1.7% and 1 : 46. Applying these rates to Swedish births in 1974, the ectopic pregnancy delivery ratio is 1 : 133.

In developing countries, ectopic pregnancy rates are generally higher than in developed countries. Kairuki[39] found a ratio of 1 : 91 for Kampala, and although direct evidence of PID was lacking, 10% to 12% of patients admitted previous treatment for gonorrhea, while 17.5% had previously had abortions. Oronsaye,[40] in a survey of 65 cases over a 3 year period in Benin, calculated a ratio of 1 : 88; 26.5% had previously had abortions while 58% had evidence of pelvic inflammation at laparotomy. Maley and Auma[41] reported pelvic inflammation in 35% of cases.

The estimates of increased risk of suffering an ectopic pregnancy following PID are about six- to tenfold the normal risk.[11, 38] In a series studied by Urquhart,[38] a ratio of ectopic pregnancy/uterine conception of 1 : 146 in controls was noted against a ratio of 1 : 19 for those who had had one attack of PID.

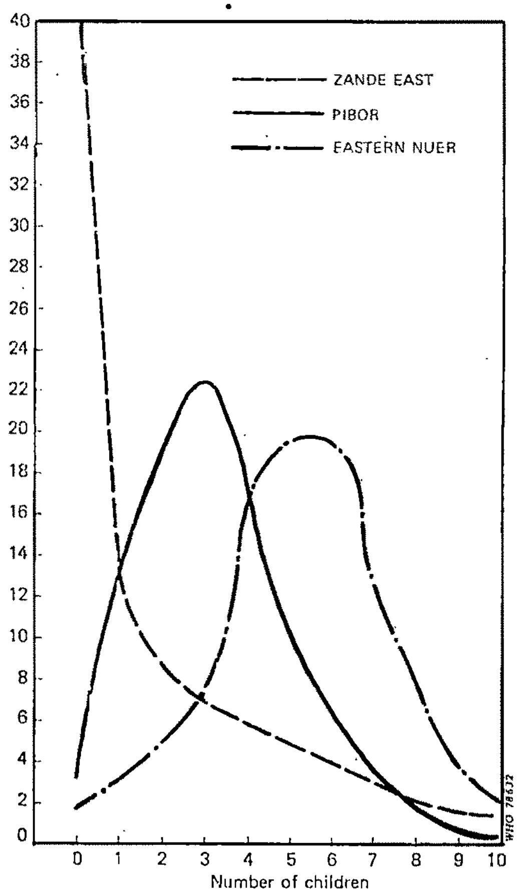

Fig. 7. Percentage distribution by number of children of women past childbearing age. Three districts in the Sudan.

An indirect indicator possibly distinguishing the role of obstetric events from STDs in the etiology of ectopic pregnancy would be the proportion of cases among nulliparous and parous women. Thus, in the two studies from East Africa, the percentages of nulliparous women among those with ectopic pregnancy were 8.7% and 9.2%.[39, 41] In contrast, in two developed countries,[42, 43] 30% and 25% of the patients with ectopic pregnancy had never been pregnant before.

Chronic PID and repeat PID. In developing countries, these two sequelae groups are likely to be extremely important. While Weström and Mårdh[11] estimate that 19.0% of individuals have a repeat acute attack through either reinfection or exacerbation of a chronic infection, it is likely that, in circumstance of low referral rates to health services for treatment of either the predisposing infection or PID itself, the rates of

Table X. Percent of infertility attributable to tubal occlusion

Author	*Country*	*Total cases*	*% Infertility owing to tubal occlusion*
Mati et al.[44]	Kenya		73.1
Campbell and Roberts-Thompson[62]	Papua New Guinea		86.4
Ratnam et al.[63]	Singapore		11.7
Dor et al.[64]	Israel		16.3
Carty et al.[60]	Kenya		60-80
Moutsinga[37]	Gabon		49
Merle and Peuch-Lestrade[65]	Cameroon		88
Allard[66]	Congo	578	64
Krishna et al.[4]	India	697	26
Meuwissen[67]	Ghana	398	61.6
Gargouche and Maximer[68]	Tunisia	114	40.4
Nakamura et al.[69]	Brazil	1,000	34.9

Table XI. Ectopic pregnancy ratio and rate following delivery and abortion, Stockholm 1972-75

Previous event	*Live births*	*Current outcome, ectopic pregnancies*	*Rate**	*Ratio*	*Births in 1974*	*Expected ectopic pregnancies*	*Ratio for multiple pregnancy only*
Delivery	3,394	50	1.2%	1:68			
Abortion	648	14	1.7%	1:46			
First birth order					48,980	124†	
Second birth order					60,894	730	1:133
Total births					109,874		

With sixfold increase, ratio among post-PID patients = 1:22.

*All pregnancies excluding induced abortion.

†Expected deliveries with previous abortion = 15.5% and percentage of these among multiparous women = 43% (does not include patients with ectopic pregnancy and nulliparous patients with PID).

production of chronic infection and reinfection will be high. Grech and associates[6] state that at Mulago Hospital, Kampala, 52% of patients with acute PID give a history of previous episodes of the disease.

With each repeat attack, the risks of suffering an ectopic pregnancy, tubal occlusion, chronic pain, and chronic infection increase. Relative risks have not been established except for tubal occlusion for which incidence rates after one, two, and three attacks are 12.8%, 35.3%, and 75%, respectively.[8, 11]

A model of PID and its consequences

In the absence of data on the prevalence or incidence rates of PID, its precursors, or its sequelae, the model developed by Weström and colleagues,[8-11] Falk,[17] Wright,[7] and others, and modified by us, may be used to test alternative hypotheses on the relative importance of the STDs, puerperal sepsis, etc., with respect to PID and its consequences.

The model assumes a predictive ability. For example, given a certain rate of ectopic pregnancy within a community, it should be possible to calculate what proportion of that is due to PID, postpartum sepsis, etc. Conversely, given that we might not know the levels of PID, but that we know the incidence of a precursor such as gonorrhea and of a sequela such as infertility, we might be able to suggest to what extent PID plays a role in the incidence of that sequela.

The incidence rates of the precursors of PID and the risks of the various sequelae following PID have been derived from studies in the United States and Sweden.

Certain subpopulations are subject to increased risks; the model can be applied to each individually. Thus, in women aged 15 to 29 years incidence rates of PID are significantly greater than in those aged 30 to 44 years.[7-11, 17] In addition, women who wear an IUCD are at significantly greater risk of developing PID and ectopic pregnancy than those who use other contraceptive methods.[11, 26, 38]

Accepting the limitations of trying to apply rates applicable to the developed world to those conditions prevailing in the developing world, it is possible that a model such as this may be used to predict at least the minimal levels given, for instance, the incidence of gonorrhea, ectopic pregnancy, or infertility.

By example, using available data from Kenya, we can compare the estimated and observed frequencies of infertility and ectopic pregnancy, as noted in Tables XIIA and XIIB.

The model predicts higher ectopic pregnancy rates,

Table XIIA. Kenya, infertility

	*Developing country rate**	*Developed country rate**	*Expected frequency**	*Comments*
Population at risk	2,826,447			
Incidence of lower genital tract gonorrhea infection (women aged 15-44 yr)	1.33%†		37,692	
Incidence of acute PID in gonorrhea population of women aged 15-44 yr	NA	10.8%	4,060	
Incidence of lower genital tract infection other than gonorrhea (ages 15-44 yr)		Unknown		
PID from other lower genital tract infections	40:60-ratio GC/other		6,090	
Total PID			10,150	Annual incidence of 0.36%
Recovery without sequelae	NA	63.4%	6,435	
Incidence of ectopic pregnancy in PID population	NA	6× Risk increase	1,274	See Table XIIB
Incidence of tubal occlusion in PID population	NA	12.8 (1)‡ 35.5 (2) 75.0 (3+)	1,299 (1) 685 (2) 1,984	Over 30 year period = 29,520
				Observed indices of infertility No. of childless women aged 44+ yr = 31,147 No. of uniparous women aged 44+ yr = 33,762 64,909
Proportion of infertility associated with tubal pathology	70%			No. associated with tubal pathology 45,436
Incidence of chronic pain in PID population	NA	18.1	1,837	
Incidence of repeat acute episodes	NA	19.0	1,929	

Legend: NA, not applicable; GC, gonococci.

*Source of estimates: Weström et al.,[8-11] Carty et al.,[60] and Mati et al.[44]

†Total adult population rate = 2%, with male: female ratio = 2:1.

‡PID episode.

i.e., 1:109, than found by Maley and Auma[41] who derive an ectopic pregnancy/delivery ratio of 1:132. Among the explanations of the discrepancy between the observed and expected rates are either underreporting or underascertainment of ectopic pregnancies or differences in the model in the setting of the developing country. For conditions such as ectopic pregnancy in the urban setting of Nairobi, gross underreporting or underascertainment seems to be an unlikely explanation.

The damage resulting from PID may be seen as a continuum: chronic pain, patent but damaged fallopian tubes giving rise to ectopic pregnancy, complete obstruction, and/or chronic lesions. In the absence of adequate health service coverage or its underutilization, the progression of the pathologic lesions following PID, with fewer cases of ectopic pregnancy but more cases of infertility, may be greater than might be expected from the model. Remember, the model is based on data from a developed country with full health service coverage and high levels of utilization.

The predictions of tubal occlusion after first and repeat attacks of PID account for only 45% of the estimate of primary and secondary infertility, if we accept the percentage of childless women more than 44 years of age as an indirect indicator of primary infertility and the numbers of uniparous women in this age group as the indicator of secondary infertility. However, since 30% of cases of infertility are associated with conditions other than tubal occlusion,[44] only 35% of cases of tubal occlusion are unaccounted for on the basis of the STD portion of the model. Presumably these cases are the result of tuberculosis, postpartum sepsis, or postabortion sepsis. This figure is not incompatible with the estimate by Grech and co-workers[6] that 19.7% of PID cases occurred post abortion or post partum. Such a model requires further testing in developing countries in order to distinguish between the different underlying causes and subsequent consequences of PID, particularly since the health strategies to prevent the consequences are quite different depending on the underlying causes.

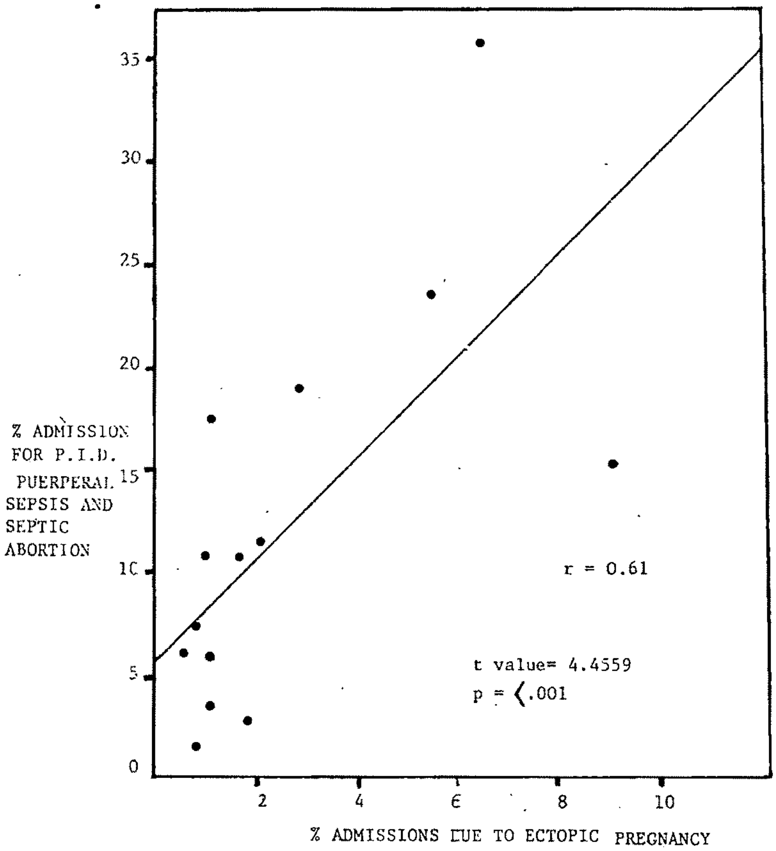

Fig. 8. Correlation of percent gynecologic admissions owing to acute PID, puerperal sepsis, and septic abortion with percent admission for ectopic pregnancy.

Table XIIB. Kenya, ectopic pregnancy

Derivation of incidence of ectopic pregnancy in post-PID population	*For non-PID population, multiparity only*	*For post-PID population, multiparity only*
Fertility rate = 218% Total female population aged 15-35 yr = 1,706,247 No. PID attacks/yr = 10,150 Total post-PID population (aged 15-35 yr) = 203,000 Total non-PID population = 1,503,247	No. births/yr = 327,707 No. multiparous (80%) = 262,166 Risk (adjusted)* = 0.75% No. ectopic pregnancies = 1,966	21% Reduction in fertility No. multiparous births/yr = $\underbrace{0.8\left(\frac{\text{population} \times \text{fertility}}{1{,}000}\right)}_{X} - 0.21\ (X)$ = 28,322 Risk increase = 6× Risk = 4.5% No. ectopic pregnancies = 1,274

Note: Total ectopic pregnancies, 1,966 + 1,274 = 3,240; ratio of ectopic pregnancy/delivery = 1 : 109.

*Derived from overall rate of 1.2% in multiparous women and allocation proportion of 6 : 1 for PID/non-PID contribution to the overall rate.

As another example, if the model is applied to the situation in the Equatoria region of Sudan, can it account for a 21.2% prevalence of childlessness among women more than 44 years of age?[45]

To account for such high levels of primary infertility would require at least a 2.1% annual rate of tubal occlusion before intended childbearing begins, i.e., for 10 years. If *N. gonorrhoeae* accounted for one half of the PID cases, then the incidence rate of *N. gonorrhoeae* among women would be 200%, a level so far seen only in Greenland. Although STD theoretically could account for such levels of infertility, it would appear that other factors are operating, either in conjunction with or independent of the STDs.

Modawi[31] examined the differential fertility levels of several Sudanese tribes and attributed the low fertility in the Equatoria area to a high prevalence of filarial infection, affecting both male and female reproductive capacities through its effect on genital lymphatic drainage. Furthermore, an examination of infant mortality rates in the area shows that this, too, can account for a high percentage of childlessness.

Support for a multiple etiologic model of PID and its consequences comes from data supplied by hospitals in 11 developing and four developed countries (Table IB and Fig. 8). Despite the variation in criteria of diagnosis and admission and the variation in the catchment area of individual hospitals, there is a significant correlation between the percentage of admissions for ectopic pregnancy and the sum of percentage admissions for PID, puerperal sepsis, and septic abortion. No correlation can be drawn between these factors individually and percent admissions for ectopic pregnancy.

In summary, this report on epidemiologic aspects of PID, its precursors, and its consequences has demonstrated that PID is a major public health problem in many areas of the developing world. The underlying causes and the type, severity, and incidence of sequelae may vary widely between countries. Epidemiologic investigations of PID, its precursors, and its consequences are essential to the implementation of health programs to combat the problem, particularly since the health strategies for prevention and control of PID will depend on the underlying causes. Derived from data from developed countries, the outline of an epidemiologic model has been presented for testing in developing countries and as an adjunct to epidemiologic research.

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Gonorrhea and female infertility in rural Uganda

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We studied sociocultural and medical factors in the Teso District in eastern Uganda and the Ankole District in western Uganda to discover the cause of the low and high fertility rates in these respective districts. The findings related to gonorrhea in women are presented in this article. Gonococcal cervicitis and clinical evidence of salpingitis occurred significantly more frequently in Teso (18.3% and 19%, respectively) than in Ankole (2.4% and 5.9%) women. Significantly more of the husbands of the gonococcal-negative infertile women compared to gonococcal-negative fertile women were found to have active gonorrhea, a past history of urethral discharge, and bilaterally thickened epididymides. (AM. J. OBSTET. GYNECOL. 138:929, 1980.)

HIGH LEVELS OF involuntary infertility are known to exist in certain countries of Africa south of the Sahara,[1] but the evidence so far available is fragmentary.[2] Concerned over the problem, the World Health Organization[3] recently designed research studies on the prevalence and the importance of various causes of infertility and pregnancy wastage.

Considerable variation in population growth rates has been known to exist for some decades in parts of Uganda. The natural increase in 1969 ranged from 2% to 3.6%.[4]

Recent studies have confirmed a high prevalence of gonorrhea in parts of urban and rural Uganda.[5-7] Romaniuk,[8] in her review of infertility in tropical Africa, concluded that the data available were not sufficient to incriminate a specific venereal disease and that further research was needed on an interdisciplinary basis to include the social and demographic factors. Considerable variation in the geographic distribution of human fertility in Uganda provided a unique opportunity to study the various factors that may be related to differential infertility rates.

Material and methods

The Uganda Demographic Research Project and sampling procedures have been described elsewhere.[9] Two districts, Teso with low fertility, in eastern Uganda, and Ankole with high fertility, in western Uganda, were selected. The crude birth rates for each district in 1969 were 37 and 55 per 1,000, respectively.[4] The diseases studied were: malaria, nutrition, gonorrhea, and syphilis. The results for gonorrhea are presented briefly in this article. The gonorrhea methodology adopted in this survey has been described elsewhere.[10] Briefly, for women it included genital examination and endocervical smears and cultures. Material for the latter was inoculated onto chocolate agar. Duplicate specimens were collected on charcoal swabs and dispatched to the laboratory in Stuart's transport medium. Husbands of gonococcal-negative fertile and infertile women also were examined for the presence of gonorrhea and evidence of infection in the past to establish their "gonococcus status." Demographic and fertility variables were recorded for each patient. The data were analyzed with a computer using the Statistical Package for the Social Sciences. Tests of significance were performed using the chi-square method with Yates' correction.

This study was sponsored by United States Agency for International Development and the Population Council, New York.

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Table I. Marital status and fertility in women in the Teso and Ankole Districts, Uganda

	No. in Teso (%)	*No. in Ankole (%)*	*Significance of difference (P)*
Marital status:			
Total	343	250	
Single	19 (5.5)	21 (8.4)	—
Currently married	263 (76.7)	188 (75.2)	—
Divorced, separated, or widowed	61 (17.8)	41 (16.4)	—
Married more than once	106 (32.7)	22 (9.6)	<0.001
In a polygamous marriage	159 (49.1)	102 (44.5)	—
Fertility:			
Total never pregnant	64 (19.7)	5 (2.2)	<0.001
Never pregnant			
Aged 20-29 yr	12/73 (16.4)	2/72 (2.8)	<0.02
Aged > 49 yr	18/85 (21.2)	0/37 (—)	
Average no. of children	2.9	5.2	

Table II. History, physical examination, and laboratory results in women in the Teso and Ankole Districts, Uganda

	No. in Teso (%)	*No. in Ankole (%)*	*Significance of difference (P)*
Lower abdominal pain	84/336 (25)	22/246 (8.9)	<0.001
Examined genitally	295	168	
Bartholinitis	7 (2.3)	—	
Purulent vaginal discharge	56 (19)	17 (10)	<0.02
Cervicitis	90 (30.5)	21 (12.5)	<0.001
Salpingitis	56 (19)	10 (5.9)	<0.001
Tender mass	23 (7.8)	1 (0.6)	<0.01
Gonococci in cervical smear or culture	54 (18.3)	4 (2.4)	<0.001

Results

Teso vs. Ankole. Three hundred and forty-three women in Teso and 250 in Ankole underwent medical examination (a response rate of 90% and 80% respectively). Forty-eight women in Teso and 82 in Ankole did not undergo genital examination. Most of these women were in menopause.

Marital status and fertility (Table I). Comparisons between the two districts with respect to various fertility indices convincingly reveal the significantly lower fertility status of the Teso women.

Clinical and laboratory aspects (Table II). In the Teso District, 84 (25%) of the women, as compared with 22 (8.9%) in Ankole, complained of lower abdominal pain (difference is significant: $P < 0.001$). Seven women in Teso but none in Ankole had signs of bartholinitis. Mucopurulent discharge in the vagina was found in 56 (19%) of the Teso women as compared with 17 (10%) of the Ankole women ($P < 0.02$). We found that 90 (30.5%) of the women in Teso but only 21 (12.5%) women in Ankole had macroscopic cervical changes, i.e., an "eroded" and/or infected cervix ($P < 0.001$). Evidence of salpingitis was elicited in 56 (19%) of the Teso women as compared with 10 (5.9%) Ankole women ($P < 0.001$). A tender adnexal mass was felt in 23 (7.8%) of the Teso sample but in only one (0.6%) in Ankole. Among the women in Teso, 54 (18.3%) had a positive cervical smear or culture for gonorrhea but only four (2.4%) in Ankole had similar positive tests ($P < 0.001$). Evidence of pelvic inflammatory disease was present in 17% of the infected Teso women. None of the infected Ankole women, however, had PID.

Gonorrhea and related findings (including thickened epididymides) in men also were found to be significantly more common in the Teso district, and have been described elsewhere.[10]

Fertile vs. infertile Teso women (Tables III and IV). Symptoms and signs possibly related to gonorrhea and PID, such as lower abdominal pain, vaginal discharge, and lower abdominal tenderness, were more prominent among those who had never conceived than among those who had experienced at least one pregnancy. Cervical secretions showed gonococci in only 10% of the infertile women as compared with 23% of the fertile women. However, as Table IV shows, 24.5% of husbands of the gonococcal-negative infertile women as compared with 6.7% of husbands of the gonococcal-negative fertile women were found to have active gonorrhea ($P < 0.01$). In this group 75.5% and 57.7% of husbands, respectively, had a past history of

Table III. History, physical examination, and laboratory results in fertile and infertile women, aged 20 to 49 years, Teso District, Uganda

Selected parameters	*No. never pregnant (%)*	*No. pregnant or with history of pregnancy (%)*	*Significance of difference (P)*
Lower abdominal pain	22/33 (66.7)	73/173 (45.1)	<0.05
Vaginal discharge	7/33 (21.2)	12/173 (6.9)	<0.05
Lower abdominal tenderness	10/33 (30.3)	30/173 (17.3)	—
Gonococci in smear or culture	3/30 (10.0)	38/165 (23.0)	—

Table IV. Gonococcal status of husbands of gonococcal-negative fertile and infertile women, Teso District, Uganda

Husbands	*No. gonococcus-negative women*		*Significance of difference (P)*
	Fertile	*Infertile*	
Gonococcus positive	9/135 (6.7%)	12/49 (24.5%)	<0.01
Past history of urethral discharge	78/135 (57.7%)	37/49 (75.5%)	<0.05
Bilaterally thickened epididymides (aged ≥30 yr)	6/103 (5.8%)	7/38 (18.4%)	<0.05

urethral discharge ($P < 0.05$), while 18.4% and 5.8%, respectively, had bilaterally thickened epididymides ($P < 0.05$).

Discussion

Our data convincingly illustrate the differences in fertility rates in the two districts, Teso and Ankole. The prevalence of PID in the two districts is stikingly different and correlates completely with the difference in fertility. Thus, our data support other studies[1, 11] that demonstrate the association between PID and infertility. Our data also suggest that gonococcal infection is common and highly correlated with both PID and infertility. A high gonococcal isolation rate among both fertile (23%) and infertile (10%) women confirms the high prevalence of gonorrhea in the Teso District. Conversely, the low prevalence of gonorrhea (2.4%) in Ankole is associated with a significantly lower prevalence of PID and higher fertility rates. In Teso, the less frequent demonstration of gonococci among infertile women was not surprising because gonococci are hard to find in the secretions of women in this late stage.[12] The contribution of gonococcal infection to infertility is substantiated further by the presence or past history of gonococcal infection among the husbands of both fertile and infertile women within the Teso District. Grech and associates,[13] and Carty and colleagues,[14] in studies of hospitalized patients in Uganda and Kenya, respectively, also demonstrated that the gonococcus was the predominant pathogen in acute PID. Unfortunately, we were not able to investigate the possible etiologic roles of *Chlamydia trachomatis*, which is now being recognized increasingly as an important etiologic agent of PID,[15] or the normal vaginal flora, which is implicated as a secondary invader of fallopian tubes.[16]

Weström[11] has shown that tubal occlusion and, thus, infertility increase with increasing episodes of PID. In the Teso District of Uganda (and possibly elsewhere in developing countries), a constant high prevalence of gonorrhea coupled with virtually no treatment[10] provide ample opportunity for repeated infection, repeated episodes of PID, and subsequent infertility.

Our findings support the conclusion that gonorrhea is an important determinant of PID and subsequent low fertility in the Teso District of Uganda.

The authors thank: The Uganda Government for permission to carry out the project, Dr. Pauline O'Neill of St. Thomas' Hospital, London, for carrying out the serologic tests, Mr. A. D. Goddard of the Computer Laboratory, University of Liverpool, for his help and cooperation in the use of computer facilities, Little Sister Lidwina of the Mulago Hospital, Kampala, for technical assistance, and all the acknowledged collaborators in a previously published work.[10]

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An overview of infectious agents of salpingitis, their biology, and recent advances in methods of detection

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NOT UNTIL THE mid-nineteenth century was salpingitis recognized as a separate disease entity. At first, tubal infections were considered as part of a generalized inflammatory process involving all pelvic organs. In 1862, Wirchow proposed the concept "perimetritis" and discussed a number of possible causes of this condition. In the 1860s and 1870s, evidence was presented suggesting that *Neisseria gonorrhoeae* might be an etiologic agent of salpingitis. In 1886, the Swedish gynecologist Westermark was the first to isolate gonococci from the fallopian tubes of a patient with signs of salpingitis. Later, cases of tubal infections were often subdivided into tuberculous and nontuberculous salpingitis, and the latter condition into gonococcal and nongonococcal salpingitis.

Although a number of assumed etiologic agents of acute salpingitis (pelvic inflammatory disease) have been considered during the twentieth century, it was not until the 1970s that more extensive research into the etiology of this infectious disease was performed. During recent years the development of new laboratory diagnostic methods and the detection and taxonomic differentiation of new organisms, such as strictly anaerobic bacteria, chlamydiae, and mycoplasmas, have contributed to our understanding of the etiology of acute salpingitis (AS). The concepts AS and PID will be used synonymously in this article.

Nontuberculous salpingitis

Epidemiologic aspects. AS is a common condition[20, 115] which is on the increase in many countries as exemplified by studies from Lund, Sweden.[135] In the United States, a slow increase has been reported in the cases of PID requiring hospitalization during the 1970s.[87] This increase in the incidence of PID seems to be associated with the pandemic situation of gonorrhea and other sexually transmitted diseases (STDs) that has prevailed during the last two decades. Most cases of AS are caused by an infection ascending from the cervix. In a large percentage of all women who develop salpingitis, the cervix is colonized by organisms known to be spread by sexual intercourse. Apart from being caused by ascending spread of STD agents, tubal infection can occur as an "iatrogenic" complication, e.g., after legal abortion, curettage, various diagnostic procedures, and insertion of an intrauterine device. The extension of practice of such measures might have contributed to the increasing incidence of AS. In this "nonvenereal" type of salpingitis, the infection of the fallopian tubes generally seems to stem from an endogenous infection, viz., caused by bacteria of the indigenous flora of the lower genital tract.

It seems reasonable to assume that the epidemiology and etiology of PID can differ from one area to another. In geographic regions with a high prevalence of tuberculosis and in parts of the world where tropical diseases are endemic, the etiologic pattern probably differs from that in areas where such diseases are seen infrequently or not at all. An important factor for the outcome of etiologic studies on PID seems to be the criteria applied in the diagnosis of the disease and in the selection of the clinical material studied. For

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This investigation was supported by grant 16X-4509 from the Swedish Medical Research Council.

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example, in patients with severe AS, the spectrum of microorganisms that can be isolated differs from that in milder cases. Delays by both patients and doctors might influence the result of etiologic studies, because the organisms that can be isolated from the internal female genital organs might change during the course of the disease. Indirectly correlated to the selective process is the awareness of the risk of sequelae, such as infertility, among women in the population studied. This may be related to the social structure of the community, or subcommunity, to which the patient belongs, as well as to the health care setup available in that community. The ratio of primary/repeated tubal infections in the clinical material studied also will influence the results of etiologic studies on salpingitis. The sampling technique, the method of transportation, and laboratory diagnostic methods used also might have such an influence.

It is my belief that the aforementioned factors, to a large extent, explain the differences in prevailing opinions regarding etiologic agents of acute AS and the relative importance of different etiologic agents of this condition.

Various workers considered the following microbes to be the major etiologic agents in nontuberculous PID: *Neisseria gonorrhoeae*, anaerobic bacteria (often in mixed infections with facultative anaerobes), *Mycoplasma hominis*, and *Chlamydia trachomatis*. These agents will be considered in detail in this article. A number of less common etiologic agents in PID will be mentioned also.

Neisseria gonorrhoeae.

Culture studies. N. gonorrhoeae is an important etiologic agent of AS.[108] As mentioned, cases of nontuberculous salpingitis often have been divided into two groups, viz., "gonococcal" and "nongonococcal" salpingitis, generally on the basis of the result of cultures for gonococci of cervical (urethral and rectal) specimens. The reported ratio of "gonococcal" vs. "nongonococcal" cases among AS patients has varied, not only in studies from different areas but also with time in one and the same hospital. Thus, approximately 50% of all salpingitis patients treated in Lund in 1960 had cervical gonorrhea, whereas during the years 1973-74 only approximately 10% of the salpingitis patients had such an infection. During the last two decades, despite a decrease in the number of salpingitis patients with gonorrhea, the total number of patients with salpingitis treated in Lund has increased steadily. In recent studies from the United States, the following percentages of cervical carriers of *N. gonorrhoeae* among salpingitis patients have been reported: 44%,[21] 50%,[116] 54%,[14] 65%,[11] and 65%.[77] Similar percentages in studies on salpingitis patients in Europe have been lower: 5%,[100] 22%,[84] 26%,[85] 27%,[104] and 32%.[19]

It has been a common finding in salpingitis patients with cervical gonorrhea that gonococci are recovered less often from the cul-de-sac and/or the fallopian tubes than from the cervix. For example, Eschenbach and associates[21] isolated gonococci from specimens collected by culdocentesis in seven of 21 women with PID and cervical gonorrhea. In the study by Chow and coworkers[11] the figure was one of 13; in that of Cunningham and colleagues[14] it was 30 of 56; and Chow and his group[12] reported a figure of three in 20 in another study. Gonococci have been recovered less often from tubal specimens of such patients than from cul-de-sac specimens. Mårdh and associates[62] reported 19 positive tubal cultures in 146 salpingitis patients with cervical gonorrhea. Sweet and co-authors,[116] who compared cultures from the cul-de-sac and the tubes of 13 salpingitis patients with positive cervical cultures for *N. gonorrhoeae*, found gonococci in the cul-de-sac of eight but in the fallopian tubes of only five of them.

It has been argued that although gonococci cannot be recovered from the tubes or the cul-de-sac, *N. gonorrhoeae* is still the causative agent in many AS cases.[14] A number of reasons for gonococci not being recovered from the fallopian tubes or the peritoneal cavity in these cases have been put forward. Gonococci have been indicated to be short-lived in the tubes and in the cul-de-sac. A higher rate of recovery of gonococci earlier vs. later in the course of the disease has been demonstrated in cul-de-sac cultures.[47] The gonococci may have succumbed, and by the time the patient is examined and sampling is performed, only a superinfection with other organisms—often anaerobes and sometimes facultative anaerobes—may be demonstrated. It also has been supposed that gonococci can penetrate the tubal mucosa and there remain undetected in attempts to recover them by endotubal swabbing. In the preantibiotic era when Curtis[16] undertook etiologic studies on salpingitis, it was customary to wait at least 2 weeks until the body temperature and the leukocyte blood count had normalized before operative procedures, including extirpation of the tubes, were undertaken. Therefore, the situation was quite different from that of present-day therapy for AS. In such subacute or chronic cases, Curtis was able to recover gonococci in homogenized tubal tissue in 19 of 64 patients with gross lesions indicating tubal inflammation, but in none of 38 patients in whom histologic examination alone could demonstrate active inflammation. There were no data on attempts to recover gonococci from swab specimens collected from the tubes, the cul-de-sac, or cervical specimens. Curtis was unable to demonstrate other organisms in the tubes from which he recovered gonococci. It may be noted that among those species of bacteria he was able to recover from the tubes of other patients in his series, no bacteria generally known as typical contaminants were detected (see also discussion of "Anaerobic infections").

Tissue homogenates are known to contain several bactericidal substances, such as lysolecithin, which in studies in vitro has been shown to possess a potent antimicrobial activity.[55] Therefore it might be questioned à priori whether tissue homogenates are particularly suited for the isolation of fastidious organisms such as gonococci. However, Studdiford and colleagues[112] were able to isolate gonococci from tubal tissue in 67% of 24 patients from whom purulent exudate did not yield gonococci.

Attempts to demonstrate gonococci in crushed fimbrial tissue specimens and tubal pus by immunofluorescence tests were not more successful than culture studies on tubal pus specimens in 30 patients with AS.[50]

In untreated cases of gonococcal salpingitis, one may assume that, with the passage of time, there will be fewer gonococci present in the tubes, a condition analogous to gonococcal infections in the lower genital tract. Tubes damaged by gonocococcal infection will constitute an organ vulnerable to endogenous infections—by anaerobic bacteria, for instance. The existence of such a "compromised" organ also might explain why organisms traditionally considered as being of low virulence and often appearing as contaminants can be recovered from the tubes or cul-de-sac specimens of these patients. The same temporal change in the pattern of organisms that can be isolated from the tubes or the cul-de-sac might occur also in cases in which the primary tubal infection was caused by other STD agents, e.g., *Chlamydia trachomatis*.

Several explanations have been offered for the "disappearance" of gonococci from the fallopian tubes, e.g., with time fewer gonococci are present in the tubes and thus may be impossible to detect by the currently used culture techniques. Immunologic and other unspecific host defense mechanisms may have killed the gonococci or they may have been killed by bacterial interaction with secondary invaders. Such interaction has been demonstrated in in vitro experiments.[102] It also is known that gonococci may die during transportation to the laboratory, as was discussed recently by Chow and his associates.[12] However, no valid explanation seems to have been offered as to why gonococci in patients with "gonococcal" salpingitis survive in the cervix but succumb more quickly in the tubes.

Little attention seems to have been paid to the possibility that tubal infection in salpingitis patients with cervical gonorrhea could be caused by other STD agents. Mårdh and co-workers[50] demonstrated, however, that chlamydiae may be found in pure culture from the tubes of salpingitis patients with cervical gonorrhea.

Eschenbach and Holmes[22] found no evidence to confirm gonococci as the cause of tubal infection in gonococcal cervical culture–negative cases.

In culture studies in gonococcal salpingitis, one ought to be able to isolate either gonococci from the tubes in pure culture or in admixture with other organisms, or alternatively other detectable organisms excluding gonococci, all of which is dependent on the time of sampling after the initial gonococcal invasion of the tubes. For ethical reasons, there, of course, have been no consecutive culture studies of specimens collected from the tubes of salpingitis patients not given antibiotics that might have proved this hypothesis. However, anaerobes are found more commonly in cul-de-sac specimens of women who have had repeated episodes of salpingitis than in those having their first attack of this disease. This observation would seem to support the hypothesis that anaerobes more easily invade a previously damaged tube than a healthy one.

As already discussed, differences in the patients studied probably explain much of the divergence of opinion on the relative importance of *N. gonorrhoeae* as an etiologic agent in "acute" salpingitis.

Serologic studies. Mårdh and associates[50] compared four serologic techniques for the detection of antibodies to *N. gonorrhoeae* in patients with complicated gonococcal infections. Radioimmunoassay (RIA)[9] and indirect hemagglutination assay (IHA)[91] for gonococcal pili antibodies were found to afford the greatest diagnostic help. However, in some salpingitis patients with cervical gonorrhea and positive cultures of gonococci, also from the fallopian tubes, these tests did not detect gonococcal antibodies. The gonococcal complement fixation test, although highly sensitive, had too low a specificity to be of use in studies of salpingitis patients.[50]

Eschenbach and his group[21] tested serum specimens of 63 salpingitis patients for the presence of RIA gonococcal pili antibodies and found such antibody activity in 84% of the 38 patients who had cervical gonorrhea and in 36% of the 25 cervical culture–negative patients. Of those in the former group, 67% showed a significant change in their antibody activity, while this was true of only 11% in the latter group of patients. Recently, Mårdh and associates[52] studied paired serum specimens of 60 consecutive salpingitis patients in whom the diagnosis was confirmed by laparoscopy. IHA gonococcal pili antibodies,[91] at a titer of ≥40, were found in 11 of the patients, three of whom had a significant change in the antibody titer. Cervical gonorrhea was diagnosed in only four of the 60 patients, all of whom had IHA antibodies. Gonorrhea had been diagnosed earlier in two of the 60 patients.

Pathophysiologic aspects. Gonococci seem to spread to the tubes preferably canalicularly via the uterus. This also seems to be the case for most other organisms causing primary tubal infection,[24] e.g., chlamydiae (see following discussion). Experimental studies[66, 131] suggest that gonococci, after having reached the tubes, attach to secretory cells of the tubal mucosal epithelium. Thereafter, the organisms invade the tubal mucosa. Gonococcal endotoxin might contribute to the tissue damage. Such endotoxin, like endotoxins of other gram-negative organisms, e.g., *Escherichia coli,* can interfere also with the tubal mucociliary wave activity, which may slow down or cease.[60] This might impair resistance to tissue invasion.

In cases of disseminated gonococcal infection, some auxotypes seem to be more prevalent than others. Whether a similar situation applies in salpingitis or whether gonococcal strains invading the tubes might have other characteristics in common, thereby differentiating them from strains not associated with gonococcal salpingitis, are not known.

At different times, it has been estimated that 2%, 10%, 15%, and 20% of all female gonorrhea patients develop symptoms of salpingitis.[90] However, the percentage of these patients in whom the tubal infection de facto is caused by gonococci is not known. Factors promoting the spread of gonococci from the cervix to the tubes are poorly understood. Recently, it has been suggested that the ability of gonococci to attach to epithelial cells of the genital tract might undergo change during the menstrual cycle,[27] which would explain why abdominal pain indicating tubal infection begins most frequently (in approximately two thirds of all cases) within a week of menstruation.[25] Other explanations for onset following menstruation have been offered, such as reflux of menstrual blood carrying gonococci to the tubes and the loss of the cervical mucus plug. The onset of abdominal pain in nongonococcal PID has proved to be distributed more evenly throughout the menstrual cycle.[20]

Anaerobic infections.

Culture studies. Strictly anaerobic bacteria frequently have been associated with acute PID, particularly in reports from the United States.[11, 14, 21, 77, 116, 119] Various species of anaerobic bacteria have been isolated from specimens collected by culdocentesis or at laparotomy from the cul-de-sac, the tubes, or pelvic abscesses. The spectrum of species recovered from such specimens has included those found to be most common in anaerobic infections elsewhere in the body, e.g., species of *Bacteroides, Clostridium, Peptococcus,* and *Peptostreptococcus.*

Several reports have stressed the significance of anaerobic bacteria in PID, particularly in cases of clinically severe infection. Thus, Pearson and Anderson[86] isolated bacteria of genus *Bacteroides* from adnexal abscesses, and Thadepalli and associates[119] recovered anaerobes in all of 16 cases of pelvic or ovarian abscess. Most commonly isolated were species of the genera *Bacteroides, Clostridium,* and *Peptostreptococcus.* Polymicrobial growth and a concomitant infection with facultatively anaerobic bacteria were frequent findings. The patients studied were severely ill and had failed to respond to previous antibiotic therapy and surgical drainage. The spectrum of anaerobic species isolated led the authors to believe that the genital tract was the origin of the infections, because *B. oralis* and *B. melaninogenicus,* species known to occur in low numbers in the flora of the colon, were commonly isolated. These species occur in the flora of the lower female genital tract.

In a study of PID by Eschenbach and colleagues,[21] *Bacteroides fragilis* was the anaerobic species found most frequently. They pointed out that this organism is not common in the vaginal flora and they took this as an indication of noncontamination of their culdocentesis samples by the vaginal flora. Others,[30] however, found *B. fragilis,* subspecies *fragilis,* to occur commonly in the cervical flora of healthy women.

Before the introduction of modern anaerobic culture techniques, cultures of specimens from chronic tubo-ovarian abscesses often were reported sterile or yielded growth of anaerobic streptococci.[1] This author[1] demonstrated such streptococci in 22 of 23 cases of chronic tubo-ovarian abscess. The average age of the patients studied was 36 (range, 24 to 52) years and they had had low abdominal pain for 6 to 13 (mean, 25) months prior to sampling. Also, in later studies of PID cases,[14] anaerobic gram-positive cocci, viz., peptostreptococci and peptococci, were the most frequent isolates among anaerobes, *Bacteroides* species being equal, or nearly equal, in frequency of occurrence.[21]

Among anaerobes isolated from specimens collected by culdocentesis from cul-de-sac and pelvic abscesses, species often have been demonstrated that generally are considered as typical contaminants from normally colonized mucosal surfaces. This also would account for some facultatively anaerobic species demonstrated in such specimens, e.g., *Staphylococcus epidermidis,* diphtheroid rods, and *Sarcina* species. A priori, one might therefore suspect that among the bacteria that actually have colonized these sites, the reported isolates also represent "pickups." In some studies, noninfected women have served as controls to evaluate the reliability of culdocentesis as a sampling method in PID. By performing culdocentesis in such women, it recently has been estimated that approximately every third "cul-de-sac" isolate from PID patients represents a contaminant from the vaginal flora.[14] In patients with infection in the lower genital tract, as is found almost invariably in salpingitis patients, the risk of such a contamination might be even greater. Sweet and coauthors[116] compared cultures of specimens from PID patients collected by culdocentesis from the cul-de-sac with specimens obtained by direct aspiration from this site during laparotomy or laparoscopy. They found fewer species of organisms in the latter specimens. They also demonstrated a discrepancy in the number of species that could be isolated by direct aspiration from tubes and cul-de-sac in the same patients. As more species were found in the cul-de-sac specimens, they wondered if a penetration of organisms from the intestinal flora into the peritoneal cavity might have taken place. Consequently they discussed whether pelvic peritonitis might provoke an inflammatory reaction in the bowel wall or alter the bowel wall resistance mechanism, thereby allowing migration of organisms from the bowel to the peritoneal cavity. Another risk of contaminating specimens during culdocentesis is the perforation of the bowel wall by the syringe introduced into the cul-de-sac.

A discrepancy similar to that found in results of culture specimens obtained by culdocentesis and by direct aspiration from the cul-de-sac has been observed when comparing transcervical and transabdominal endometrial aspirates from women with postpartum pelvic infection.[43] This study is of interest in relation to salpingitis, because the causative agent(s) in salpingitis generally is thought to spread via the endometrium to the tubes. Falk,[24] who performed cornual resection in more than 1,000 salpingitis cases, was unable to demonstrate reinfection of the tubes in any of them during a follow-up period of more than 8 years. This strongly supports the concept of a canalicular spread of infection in the majority of salpingitis cases.

The aforementioned risks of contamination cannot, however, conceal the fact that anaerobic infections are of importance in PID. Those women with PID in Lund, from whom anaerobic bacteria were isolated from cul-de-sac specimens or pelvic abscesses, were generally more seriously ill than the average salpingitis patient and also presented with pelvic abscesses more often. This suggests that the discrepancy among studies on the etiology of AS in this town[62] and that among recent studies in the United States[11, 14, 21, 77, 116, 119] with regard to the findings of anaerobic infection may well be explained by a difference in the selection of the patients studied. As already mentioned, laparoscopy is performed routinely in Lund in cases of assumed salpingitis, many of which present comparatively mild symptoms.

Therefore, more patients probably had mild PID compared to patients seen in gynecology departments not performing visual inspection of the tubes. Thus, if anaerobes are commonly recovered from the cul-de-sac of patients with PID in a given geographic area, this might imply that only the "tip of the iceberg" of the salpingitis problem as a whole is being considered.

Serologic studies. Söderberg and his group[104] and Lindberg and associates,[46] in Sweden, used an enzyme-linked immunosorbent assay technique to detect antibodies to polysaccharide antigens of the *B. fragilis* group of bacteria. They concluded that these bacteria play a role in AS only infrequently. These results stand in contrast to those of Kasper and co-workers,[37] in the United States, who used an RIA technique to determine antibodies to capsular polysaccharide antigens of *B. fragilis* (subspecies *fragilis*) in women with acute PID. The serologic test results were compared with those of cultures of specimens collected by culdocentesis. Changes in antibody concentration in paired serum specimens from patients with gonococcal and nongonococcal PID in whom this bacterium had been isolated from the cul-de-sac were significantly greater than those in culture-negative patients. An increase in the antibody concentration occurred in five of nine culture-positive patients, while only two of the 24 culture-negative patients had such an increase. The study by Kasper and associates suggests a role of *B. fragilis* in PID patients from whom the organism can be isolated from the cul-de-sac. Their finding also seems to suggest that even though *B. fragilis* occurs most often as a secondary invader in the tubes (discussion to follow), the organism can nevertheless play a role in the early course of the disease in some PID cases.

Pathophysiologic aspects. It is common knowledge that anaerobic infections are found most commonly in "compromised" hosts and are generally of endogenous origin. In salpingitis, it is believed that anaerobic bacteria often gain entrance to the tubes as secondary invaders from the lower genital tract in patients whose tubes have been damaged by infection with STD agents. In such secondary infections, both anaerobic and facultatively anaerobic bacteria can be demonstrated.

Anaerobic infections of the tubes seem to occur more frequently in women who have had repeated episodes of salpingitis. This might support the concept that endogenous infections by anaerobes may occur in the fallopian tubes, representing a locus minoris resistenti. Endogenous tubal infections may occur in hosts whose genital organs have been "compromised" by gynecologic surgery, curettage, legal or illegal abortion, or various diagnostic procedures. In the hospital catchment region of Lund, such "iatrogenic" cases constitute approximately 15% of all salpingitis patients.[134] However, even in these patients, STD agents can be involved etiologically unless precautions are taken to diagnose and eradicate such agents prior to or concomitant with the procedures undertaken. Prophylactic treatment with nitroimidazoles, which are active against anaerobes, reduces the frequency of postoperative genital infections.[113] Whether such treatment also can reduce the frequency of anaerobic infections of the fallopian tubes following iatrogenic measures other than gynecologic surgery remains to be determined.

Mycoplasma hominis **and** *Ureaplasma urealyticum.*

Culture and serologic studies. Since the late 1930s, it has been known that mycoplasmas can colonize the lower female genital tract. For ureaplasmas, this has been known since the mid-1950s. The species isolated have been *Mycoplasma hominis, Ureaplasma urealyticum,* and (rarely) *Mycoplasma fermentans.* Since early after their initial discovery in the female genital tract, a number of reports have appeared on the isolation of large colony-forming mycoplasmas from the cul-de-sac or the internal female genital organs in cases of PID. These isolates presumably represented *M. hominis,* although *M. fermentans* was isolated from one such patient. An antibody response to *M. hominis* in patients with AS was demonstrated also. The isolation frequency and the prevalence of antibodies to *M. hominis* in PID patients have been among the highest demonstrated clinically in various groups of women.[61, 83, 117]

In a study of 50 women with salpingitis,[56-58] *M. hominis* was recovered from the tubes in pure cultures in four cases, corresponding to 13% of those harboring the organism in the cervix. No such mycoplasmas were isolated from the tubes or cul-de-sac in 34 women with assumed salpingitis, but in whom laparoscopy revealed normal tubes. In seven of 29 women with AS from whom paired sera were obtained, a significant change was found in the titer of antibodies, as studied by IHA.[39] In three of these seven women, *M. hominis* was recovered from the tubes. In a study of the serum concentrations of IgA, IgG, and IgM in 53 women with AS, 18 had markedly elevated levels of IgM, viz., 300% to 500% of that of a standard serum pool. The IgA and IgG levels were normal, however. IHA antibodies to *M. hominis* occurred in all but one of these 18 specimens. *M. hominis* was recovered from lower genital tract of 14 and from the tubes of two of the 15 patients in whom tubal specimens were studied for this organism. Five of six patients in whom a significant increase in serum antibody titer could be demonstrated had elevated serum levels of IgM.[51]

U. urealyticum also has been recovered from the cul-de-sac or the fallopian tubes of patients with acute PID. In two of 50 such cases, Mårdh and Weström[55] recovered this agent directly from the fallopian tubes. In one of these two patients paired sera revealed a significant increase in the titer of metabolism-inhibition antibodies.[59] Solomon and associates[105] and Braun and Besdine[6] isolated ureaplasmas from peritoneal fluid and a tubo-ovarian abscess respectively.

During recent years, *M. hominis* and *U. urealyticum* have been isolated from specimens collected by culdocentesis in patients with PID, that is, in all those studies of PID in which culture techniques suitable for these organisms were employed. Thus Eschenbach and colleagues[21] recovered *M. hominis* in two and *U. urealyticum* in one of 54 such specimens, while the corresponding figures in a study by Sweet and co-workers[116] of 26 women with PID were one and four, respectively. In these latter five patients, only one strain of *U. urealyticum* could be demonstrated in tubal specimens.

Eschenbach and his group[21] isolated *M. hominis* from the cervices of 145 of 204 PID patients. A significant increase in the titer of mycoplasmacidal antibodies occurred in 17 (22%) of 84 of these patients. *U. urealyticum* was found in cervical specimens of 164 of the same 204 women. In 15 (18%) of the patients also studied for *M. hominis* antibodies, a significant change in the titer of ureaplasmacidal antibodies was demonstrated. No difference between patients from whom *N. gonorrhoeae* was and was not isolated from the cervix was found with regard to the culture and serologic studies on *M. hominis* and *U. urealyticum.*

In a recent study of 60 patients with AS, 40% had IHA antibodies to *M. hominis* at a titer of ≥40. A significant increase in the titer of such antibodies occurred in 17% of the patients. Of 50 age-matched controls, 8% had such antibodies.[52] This suggests that in a significant number of cases acute PID is associated with a current infection with *M. hominis.* Current double infections with *M. hominis* and *C. trachomatis,* as indicated by the occurrence of a significant increase in the titers of antibodies to both these organisms, were demonstrated in 7% of the 60 patients studied.

Møller and associates,[75] in a study of salpingitis in Denmark, isolated *M. hominis* from the cervices of 47% of 110 patients with this disease. A significant increase in the titer of IHA antibodies was demonstrated in 29 of the 52 culture-positive women, while this was true in only four of the 58 culture-negative cases.

A discrepancy between the results of serologic studies on the one hand and culture studies on cul-de-sac and fallopian tube specimens of PID patients on the other might be explained by the fact that, in the main, *M. hominis* causes parametritis (see following discussion). Hence the two types of specimens mentioned probably are not particularly suitable for the establishment of an etiologic role for these organisms in PID.

Pathogenicity factors in genital mycoplasmas and ureaplasmas continue to be poorly understood. In tissue organ cultures of human fallopian tube epithelium infected with *M. hominis*, a cytopathogenic effect (CPE) on the tubal cilia has been observed.[63] In such organ cultures, *M. hominis* caused a rapid and marked slowing down of the mucociliary wave activity.[60] A similar CPE, which has been attributed to ammonia production,[109] was observed in bovine oviduct cultures challenged with human ureaplasmas.[110] Ammonia is also produced by *M. hominis*.

Experimental infections in monkeys. Møller and colleagues[72] were able to provoke salpingitis in grivet monkeys by inoculation of *M. hominis* into the fallopian tubes. Signs of inflammation occurred within 3 days. The tubes became swollen after 1 week, when they were also hyperemic throughout their entire length. The parametria were then moderately edematous. After 12 days, the parametria showed marked swelling. After 4 to 5 weeks, the internal genital organs were virtually normal. Biopsies of the tubes showed that the mucosa was slightly congested and there was a lymphoid infiltration. An intensive infiltration of the subserosa by lymphoid cells together with an increased number of polymorphonuclear leukocytes, often extending into the peripheral layers of the muscularis, were found. There were edema and hyperemia in the parametria as well as lymphocyte infiltration. Fat necrosis and granulation tissue appeared in the parametria after 10 to 12 days. Møller and Mårdh[74] also showed that parametritis, but not salpingitis, did occur after ligation of tubes in animals infected with *M. hominis* in the uterine cavity.

In similar animal studies, *U. urealyticum* did not produce salpingitis.[73]

Chlamydia trachomatis.

Culture and serologic studies. In 1977, Mårdh and associates[54] reported the isolation of *C. trachomatis* from the cervices of 19 of 53 consecutive patients with AS. The organism also could be recovered in pure culture from the tubes in seven of the 20 patients from whom tubal specimens were obtained. Six of these seven cervical culture-positive patients also harbored *C. trachomatis* in the tubes. In two previously published studies, *C. trachomatis* was recovered in individual PID patients from the tubes or from cul-de-sac specimens.[15, 21] Eschenbach and co-authors[21] found microimmunofluorescent (micro-IF) antibodies to *C. trachomatis* in seven of 91 women with PID, including 19 of 20 women from whom the organism was recovered in the cervix.

In a series of 143 salpingitis patients, in whom the diagnosis was confirmed by laparoscopy, 62% had IgG micro-IF antibodies to *C. trachomatis* at a titer of ≥64.[126] Of the 143 patients, 23% had IgM antibodies (titer ≥8). There was a correlation between the severity of the tubal inflammation, as revealed by visual inspection of the tubes through the laparoscope, and the geometric mean titer (GMT) of such antibodies. Thus the GMTs of IgG antibodies in the salpingitis patients who had mild, moderate, and severe inflammation of the tubes were 15, 24, and 58, respectively. In 19 women lacking signs of salpingitis, the corresponding GMT was 2. Studies on fluid collected from the cul-de-sac of 27 women with salpingitis revealed a high GMT of IgA and IgG antibodies to *C. trachomatis*, viz., 61 and 285. These latter serologic test results also were correlated to the severity of the tubal infection. Thus, in patients with severe tubal inflammation the GMTs for IgA and IgG antibodies were 90 and 1,721, respectively. Comparison of the antibody levels in cul-de-sac and serum specimens of the same patients suggests a local antibody production; titers of both IgA and IgG antibodies were higher in the former than in the latter specimens.

In a recent study of paired sera of 60 consecutive salpingitis patients,[52] IgG antibodies to *C. trachomatis* at a titer ≥32 occurred in 80%. A significant titer change of such antibodies was demonstrated in 38%. IgM antibodies occurred in 20% of the patients. This study indicates that acute infection with *C. trachomatis* is common in salpingitis patients in Lund.

Simmons and his associates,[101] in London, found that 59% of 17 patients with severe assumed AS had serum IgG antibodies to *C. trachomatis*, while IgM antibodies to this organism occurred in 30%. In patients with less pronounced clinical signs of PID, the corresponding figures were 40% and 30%, respectively.

Paavonen and co-workers,[85] in Helsinki, Finland, studied 106 salpingitis patients. Of these, 26% harbored *C. trachomatis* in the cervix. Paired sera were obtained from 72 patients. Of the chlamydia culture-positive patients, 36% had an antibody response to *C. trachomatis*, while the corresponding percentage in the culture-negative cases was 18%. The GMT of IgG antibodies to *C. trachomatis* was 219 in the former and 73 in the latter group of patients. Of the chlamydial culture-positive patients, 30% had gonorrhea, as compared with 24% of the culture-negative patients.

Recently, Paavonen,[84] in an extension of the aforementioned study, stated on the basis of clinical, culture, and serologic investigations that *C. trachomatis* is a common etiologic agent of acute PID in Helsinki. In the 228 salpingitis patients studied, mean age was 27.8 (range, 15 to 45) years. The mean duration of symptoms before examination was 6 days. Of the 228 women, 62% had been diagnosed as having less severe AS, whereas 38% had pelvic peritonitis and/or tubo-ovarian or pelvic abscesses. Of the women, 30% had positive cultures for *C. trachomatis* from the lower genital tract. Double infections with this agent and *N. gonorrhoeae* occurred in approximately one fourth of the patients. IgG antibodies (titer ≥64) to *C. trachomatis* occurred in 68%, while 24% had titers of ≥512; the GMT was 73. In the *C. trachomatis* culture-positive cases a significant titer change occurred in 32%; the GMT was 264. The corresponding figures in the culture-negative cases were 13% and 43. A correlation between severity of PID and the antibody titer also could be demonstrated. Thus, the GMT was 192 in the patients with peritonitis and/or pelvic abscess, while it was only 39 in those with mild or moderately severe salpingitis.

Patients' historical signs and symptoms in "chlamydial" and "gonococcal" salpingitis differ in several respects.[114] Thus patients with evidence of chlamydial AS generally have a longer history (>3 days) of low abdominal pain and have febrile illness more often (rectal temperature >38° C) when examined. They have fewer symptoms but a higher erythrocyte sedimentation rate when first examined, as compared to patients with "gonococcal" AS. In the group of patients with "gonococcal" AS, the onset of low abdominal pain was associated more often with menstrual bleeding than in those with chlamydial AS.

Experimental animal models. Experimental infections in grivet monkeys have produced further evidence of the ability of *C. trachomatis* to provoke salpingitis. In grivet monkeys, inoculation with a strain of *C. trachomatis*, immunotype K (isolated from the fallopian tubes of a woman suffering from salpingitis), into the tubal lumina, into the uterine cavity after curettage, or into the cervical mucosa resulted in salpingitis.[73, 74, 94] Evidence of a canalicular spread of chlamydiae causing salpingitis was obtained by experiments in which the tubes had been ligated prior to the inoculation of the organism into the uterine cavity. In these animals, salpingitis did not develop. The gross lesions and the histologic findings in tubes of monkeys infected with *C. trachomatis* were similar to those reported to be characteristic of gonococcal salpingitis in women,[16, 81, 112] viz., the tubes were swollen and reddened and the tubal epithelium was destroyed. The tubal lumina were filled with an exudate containing lymphocytes and desquamated epithelial cells, while the tubal wall was heavily infiltrated through all layers with lymphocytes and some polymorphonuclear leukocytes.

The monkey studies referred to fullfilled Koch's postulates with regard to *C. trachomatis* as an etiologic agent of AS.

In two patients[76] in whom *C. trachomatis* was isolated in pure culture from the fallopian tubes and who developed a significant antibody response to the organism (with extremely high IgG titers), the macroscopic and histologic appearance of the tubes was similar to that found in the monkeys experimentally infected with chlamydiae (and thus also similar to that in women with gonococcal salpingitis).

Follow-up studies for 2 months on two monkeys experimentally infected with *C. trachomatis*, which provoked salpingitis, revealed thickened tubal mucosal folds and adhesions in one of the animals, while in the other the tubal lumina were reduced in size and the epithelium was atrophic and flattened.[74] These findings may suggest that tubal chlamydial infections might result in impaired tubal infection and, hence, in infertility or an increased risk of ectopic pregnancy.

There still is little information on the role of the immune system in either resolution of genital infections with *C. trachomatis*, including salpingitis, or resistance to reinfection. It seems probable that infections of the lower genital tract with this agent can persist, despite the presence of chlamydial humoral and local antibodies. However, such antibodies may inhibit pinocytosis of chlamydiae in eukaryotic cells,[35] resulting in false negative in vitro cultures. In experimental studies on ocular infections with *C. trachomatis* in monkeys[65] and in guinea pigs infected with the guinea pig inclusion conjunctivitis (GPIC) agent,[67] the finding of local antibodies has been found to correlate with resistance to infection. The role of cell-mediated immunity in human genital chlamydial infections is poorly defined, although such immunity could possibly develop.[31] In preliminary studies of grivet monkeys in which salpingitis was provoked by injection of *C. trachomatis* inoculum into the tubes and by subsequent repeated injections into the cervix, a humoral (but no cell-mediated) immune response could be demonstrated.[73] Recently, White and associates[137] found that in immunosuppressed guinea pigs the GPIC agent could spread to the fallopian tubes and provoke endometritis and salpingitis, resulting in pyosalpinx and hydrosalpinx.

Immunosuppression mediated by daily doses of cyclophosphamide hindered the development of humoral and cell-mediated responses to the organism and caused prolonged genital chlamydial infection. This was also true when the humoral (but not the cell-mediated) immunity was suppressed, indicating that the former is essential for recovery from genital infections with this agent. The mode in which humoral antibodies control the intracellular parasitism of GPIC is not known; also, it is unknown whether secretory antibodies are important in this respect. In nonimmunosuppressed guinea pigs, genital infections with GPIC resolved in 20 to 25 days, and the animals were then resistant to challenge infection.[89] In nonimmunosuppressed grivet monkeys infected with *C. trachomatis*, immunotype K, in the tubes, the uterus, or the cervix, infection resolved without antibiotic treatment within 5 to 6 weeks.[73]

The diagnostic value of culture and serologic studies for C. trachomatis *in AS*. The outcome of culture studies for *C. trachomatis* in patients with AS seems to be correlated to the host's immune response to the organism prior to the time of sampling. Thus, groups led by Mårdh[32] and Svensson[114] found an inverse correlation between the titer of serum IgG antibodies to the organism and the result of cultures for *C. trachomatis* from cervical specimens. In patients with a titer <64, 75% were culture-positive for *C. trachomatis* from the cervix, whereas in patients with titers of 64, 128, 256, and ≥512, the corresponding percentages were 54%, 37%, and 18% (Fig. 1). Therefore, patient (and doctor) delays, as discussed previously, may be related indirectly to the finding of *C. trachomatis* by culture studies in PID patients.

In experimental studies, the presence of chlamydial antibodies in tissue cell cultures can hinder pinocytosis of chlamydiae and hence the formation of intracytoplasmatic inclusions in such cultures.[35] This might result in false negative chlamydial cultures when studying clinical specimens containing antibodies to *C. trachomatis*.

Treharne and co-authors[126] reported that IgM antibodies in human beings with genital chlamydial infections persist for 8 to 10 weeks after infection. Other studies[97] on patients with venereal disease suggest that such antibodies persist for a longer period of time. If this is so, the demonstration of IgM antibodies would be of limited value for the establishment of current genital chlamydial infections.

By the use of a modification[125] of the micro-IF antibody test,[128] Ripa and colleagues[94] and Møller and Mårdh[74] determined the occurrence of serum antibodies to *C. trachomatis* in grivet monkeys in whom salpingitis had been provoked by this organism. Such IgM antibodies could be detected after 1 to 2 weeks and had disappeared 5 to 8 weeks after the infection, while IgG antibodies appeared within 2 to 3 weeks but were still present after 10 to 12 weeks, when the experiments were ended.

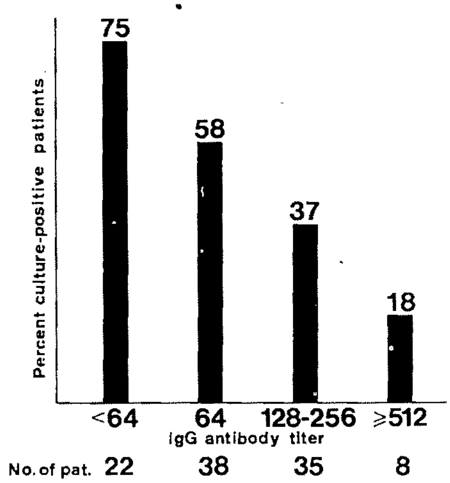

Fig. 1. Inverse correlation between serum IgG antibodies to *Chlamydia trachomatis* and result of cultures for the organism from the cervices of 60 salpingitis patients.

In two of 10 patients with salpingitis and from whom *C. trachomatis* was isolated in pure culture from the tubes, micro-IF IgM antibodies at a titer of ≥8 were found, while IgG antibodies occurred in all patients. The highest titer of IgG antibodies demonstrated was 2,048. Follow-up studies on some of these patients indicated that serum IgM and IgG antibodies to *C. trachomatis* do not persist for years in tetracycline-treated salpingitis patients. Thus when retesting two of these patients 1½ years after the first serum samples had been drawn, IgM or IgG antibodies to *C. trachomatis* could not be detected, even though high antibody titers had been detected in the previously tested sera.

Recently Schachter and associates[98] stated that tests for serum IgM and IgG antibodies and such IgA and secretory IgA antibodies in cervical secretion cannot be substituted for agent isolation when diagnosing genital chlamydial infection.

Epidemiologic aspects of genital infections with C. trachomatis. Culture studies indicate that genital colonization with *C. trachomatis* is common in young, adult women in western nindustrialized countries. For example, in southern Sweden, such infections occurred in 6.1% of a series of 231 consecutively examined pregnant women, while 8.7% of 273 puerpera women from the same area also harbored the organism. When analyzing the recovery frequency in pregnant and puerperal women by age, it was found that in women

$\geq$ 19 years of age, 10% and 24%, respectively, had positive cervical cultures. In the age group 20 to 24 years, the corresponding figures were 8.7 and 10.2; while in women beyond that age, chlamydiae occurred in 4.2% in both groups.[51]

In a seroepidemiologic study in Greenland, viz., a society with a high prevalence of venereal disease (with an approximate 100-fold prevalence of gonorrhea over the aforementioned community), evidence was found of exposure to *C. trachomatis*, immunotype D-K, which was common in that community.[49] Among other things, sera were tested from all individuals in a small settlement. IgM antibodies to *C. trachomatis* were detected in 6% of the men and 6% of the women, while IgG antibodies occurred in 21% and 51% of the total 150 individuals studied.

The aforementioned studies suggest a high prevalence of genital infections with *C. trachomatis* in women in both industrialized and developing countries. The risk of ascending spread of a cervical infection with *C. trachomatis* remains, however, to be determined.

Which data support the hypothesis that *C. trachomatis* is probably the most common etiologic agent of acute PID, at least in the Scandinavian countries? The organism is isolated from the cervices (apart from organisms constituting the normal flora) and the fallopian tubes of patients with this disease more frequently than any other agent. An antibody response to *C. trachomatis*, including a significant antibody change, does occur frequently in patients with AS, and significantly more often than responses to *B. fragilis*, *M. hominis*, and *N. gonorrhoeae*, as evidenced by the studies referred to elsewhere in this article.

Differences of prevailing opinions of the relative importance of etiologic agents of AS in the United States[13, 22] and in Sweden probably could be explained by the earlier discussed differences in the selection of salpingitis patients studied.

It has been argued that since no difference in the therapeutic effect of penicillin/ampicillin and tetracycline could be demonstrated in a short-term follow-up study of PID patients, it is difficult to imagine any important etiologic role of *C. trachomatis*.[15] However, in less severe salpingitis cases, as studied by the authors mentioned last, symptoms disappeared and both erythrocyte sedimentation rate and white blood count generally returned to normal values within a couple of weeks, even without antibiotic treatment. In severe cases of acute PID with abscess formation, such a benign clinical course is not probable. Ampicillin may suppress, although probably will not eradicate, genital chlamydial infections. Thus, when sampling for *C. trachomatis* is done shortly after such a course of therapy, false negative cultures may be obtained. In long-term follow-up studies made with regard to preservation of fertility in salpingitis patients, Weström and associates[133] found no difference in salpingitis patients who were involuntarily childless and recommended the following antibiotic regimens: benzyl penicillin plus streptomycin, chloramphenicol, ampicillin, or tetracycline. This study, which seems to be unique with regard to the duration of follow-up, viz., >8 years (a time period which is necessary to evaluate the impact on fertility of antibiotic treatment of AS), stresses the need for epidemiologic measure in order to reduce the prevalence of STD agents that can cause this disease.

Due to the high percentage of asymptomatic carriers of *C. trachomatis*[97] contact tracing seems to be one important measure for the prevention of chlamydial salpingitis. The results of contact tracing of *C. trachomatis* indicate that this could be just as successful as contact tracing in patients with gonorrhea. Thus, Thelin and his group[120] found that in 103 female consorts of 112 men with *C. trachomatis* infection, 65% harbored the organism. Of 95 male partners to 85 such female patients, 53% were found to be infected with *C. trachomatis*. Half of the chlamydia-positive consorts were symptomless. In a similar study of consorts to patients with gonorrhea by the same authors, 86% of the male and 78% of the female consorts harbored *N. gonorrhoeae*. Of these consorts, 50% and 40%, respectively, were symptomless. Consorts to patients with salpingitis and cervical gonorrhea are often asymptomatic. Thus Eschenbach[23] found 41% of gonococcal carriers among such consorts to be asymptomatic.

Chlamydial perihepatitis in acute salpingitis. Perihepatitis as a complication of salpingitis was first reported by Stajano in 1919 and is now known as the Fitz-Hugh–Curtis syndrome.[17, 26] In recent studies on perihepatitis, gonococci have been recovered from the cervices of approximately half of all such patients, but seldom from peritoneal acvities.

Müller-Shoop and co-workers[79] studied 11 female peritonitis patients 17 to 38 years old, seven of whom also had signs of perihepatitis. Of the remaining four, two had salpingitis and peritonitis but no signs of perihepatitis. Ten of the 11 patients (including the two salpingitis patients) had serum antibodies to *C. trachomatis*, suggesting a current or recent chlamydial infection. Chlamydial cultures were not obtained, while cervical cultures for *N. gonorrhoeae* were positive in two of the patients with perihepatitis and in one of those with salpingitis. The laparoscopic findings in three of the 11 women revealed "violin-string" adhesions described as being characteristic of the Fitz-Hugh–Curtis syndrome. However, none of the patients in this study suffered from the syndrome, viz., salpingitis complicated by perihepatitis.

Wölner-Hanssen and associates[138] recently diagnosed perihepatitis in three women with evidence of acute chlamydial salpingitis. One of the patients was a 14-year-old girl. She underwent laparoscopy because of abdominal pain of the right upper quadrant. An exploratory laparotomy revealed salpingitis and perihepatitis. A liver biopsy disclosed a thickened, fibrinous capsule, but there were no signs of altered parenchymal architecture. Adhesions of "violin-string" appearance between the liver surface and the anterior abdominal wall were found. The patient had a significant change in both IgM and IgG (maximum titer, 4,096) antibodies to *C. trachomatis*, suggesting a current chlamydial infection. No gonococcal IHA pili antibodies were demonstrated. The history suggested a sexually transmitted infection with *C. trachomatis*. Thus, 2 weeks prior to the onset of the patient's abdominal pain, her first and only sexual partner had been treated for a chlamydial culture-positive urethritis. The second patient was an 18-year-old woman who presented with lower abdominal pain. Acute salpingitis was assumed and confirmed by laparoscopy, which unsuspectedly also revealed perihepatitis. Evidence of a chlamydial (but not of a gonococcal) infection was found. In the third case, findings were similar, but this woman had attended a surgical department because of right-upper-quadrant pain suggestive of cholecystitis. The microbiologic findings in this case suggested a current chlamydial infection. In the study a woman was described who had evidence of chlamydial salpingitis and symptoms indicative of perihepatitis, but in whom the laparoscopy revealed a normal liver surface.

Some young women (<30 years old) with symptoms suggestive of cholecystitis, who attend surgical departments and hospital emergency rooms, seem to be suffering from salpingitis complicated with perihepatitis. The study by Wölner-Hanssen and colleagues[138] indicates that right-upper-quadrant pain may occur also in patients with AS but without laparoscopic signs of perihepatitis, and that perihepatitis may occur in women with salpingitis without symptoms suggestive of this complication.

Perihepatitis and peritonitis were demonstrated in a grivet monkey infected with *C. trachomatis* in the uterine cavity after curettage.[74] The tubes of this monkey had been ligated prior to infection. There were no signs of salpingitis at autopsy performed 2 weeks after the infection. The monkey had adhesions between the anterior abdominal wall and the liver surface. There were signs of acute inflammation in the liver capsule, but there was no parenchymal involvement. This study indicates that genital chlamydial infections can spread to the liver surface by routes other than canalicularly through the tubes. As mentioned, perihepatitis (and peritonitis) in the absence of salpingitis has been found in women with serologic evidence of chlamydial infection.[79] The demonstration of a case of gonococcal perihepatitis and peritonitis with "violin-string" adhesions in a man[38] also indicates the possibility that Fitz-Hugh–Curtis syndrome may be caused by an infection not spread canalicularly through the tubes.

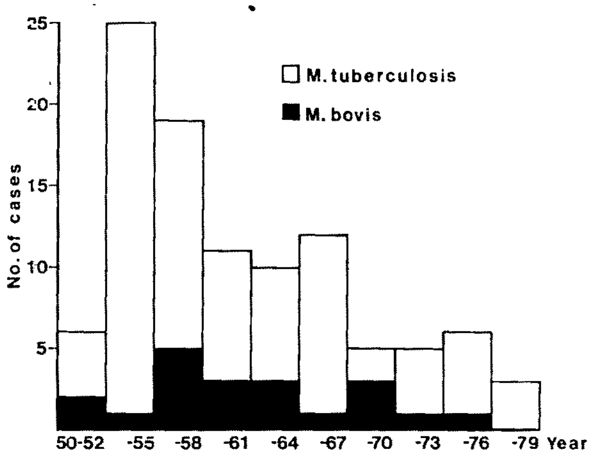

Fig. 2. Annual number of female genital tuberculosis cases diagnosed by culture studies in southern Sweden (Malmö excluded) from 1950 to 1979. The laboratory catchment region has approximately one-half million female inhabitants.

Miscellaneous, uncommon etiologic agents. Primary respiratory tract pathogens, such as group A streptococci,[78] *Haemophilus influenzae*,[4, 56] and *Mycoplasma pneumoniae*,[123] have been isolated from the tubes of patients with signs of salpingitis.

Campylobacter fetus is another rare cause of salpingitis, as evidenced by a case in which septicemia also occurred.[8] PID infections with *Actinomyces* have been diagnosed in IUD users.[10]

The observation that *Enterobius vermicularis* can invade the tubes has been made repeatedly, as recently reviewed by Saffos and Rhatigan.[95]

Among tropical diseases that may be associated with PID, filariasis and schistosomiasis have been considered. However, few data are available on the prevalence, epidemiologic pattern, and pathophysiology of these infectious diseases in PID.

Besides tuberculosis, a number of chronic infections may involve the internal female genital organs. However, lack of space precludes the consideration of such infections.

The role of viruses in acute PID still awaits thorough study. In a pilot study of 15 cases,[56] Coxsackie B5 and ECHO 6, respectively, were isolated from the tubes of two such patients. The latter isolate, however, probably represented a laboratory "pickup." Certain rhinoviruses have been able to grow only in tissue organ cultures of human fallopian tubes.* Whether this observation has a clinical counterpart remains to be established.

Tuberculous salpingitis

Nowadays, tuberculosis of the fallopian tubes seems to be a rarity in most western industrialized countries. Figures on the prevalence of this disease in developing countries seem to be meager.

In 1921, Curtis[16] found tuberculous salpingitis (TS) in 9% of a series of 102 American women with inflammatory changes in the tubes. In the early 1960s, American textbooks on gynecology stated that approximately 5% of all salpingitis cases in developed western countries were associated with mycobacterial infection.[81]

Fig. 2 shows the number of cases of genital tuberculosis diagnosed by cultural studies in women in southern Sweden (Malmö excluded) during the years 1950 through 1979. The total number of such cases during these 30 years was 102. The female population of the laboratory catchment region is roughly one-half million women. As seen in the figure, the number of diagnosed cases of genital tuberculosis tended to decrease during the study period. Although no figures are available exclusively for genital tuberculosis, tuberculosis of the urogenital tract in Swedish women during the last decade has accounted for 5% to 10% of all diagnosed cases of tuberculosis.

It is notable that 19.6% of the isolated mycobacterial strains belonged to *Mycobacterium bovis*. The remaining strains were diagnosed as *Mycobacterium tuberculosis*. Because cattle in the study area have been free of tuberculosis during the last three decades, the culture results suggest that the patients from whom *M. bovis* was isolated must have acquired their infections long ago.

The mean age of patients with TS in the community studied is much greater than that of patients with nontuberculous PID. The eradication of bovine tuberculosis from an entire region will be accompanied by a decrease in the number of cases of genital tuberculosis in women.

In women with tuberculous peritonitis, or pelvic tuberculosis, the tubes are involved almost invariably. Tuberculous salpingitis also can occur, however, in women with no signs of generalized peritonitis.[81]

It has been demonstrated that TS generally is associated with pulmonary tuberculosis, although often only a healed primary complex can be found. A hematogenic spread with a predilection for the fallopian tubes seems to occur in such patients. The occurrence of tubal infections with *M. bovis* suggests that there might also be primary foci other than the lungs. Tuberculosis of the tubes is seldom considered to be a sexually transmitted infection, although, in principle, a woman can be infected by a man with tuberculous epididymitis.

The gross lesions of the tubes in cases of tuberculosis can be similar to those in recurrent, chronic gonococcal salpingitis.[16] Thus, the diagnosis of tuberculous salpingitis in Curtis' nine cases could not be diagnosed in the operating theater, but only after careful examination of the extirpated tubes and in three cases only after histologic examination.[16] Often a thorough microscopic examination of the tubes must be made before the diagnosis can be confirmed. The occurrence of tubercles can be limited to the mucosa; they seldom occur in all layers of the tubes. There may be a proliferation of the mucosal folds resembling that found in cases of adenocarcinoma.[81] In patients with Crohn's disease, histologic changes of the tubes resembling those found in TS have been demonstrated recently.[7]

Obtaining specimens in AS

Lower genital tract. In addition to a cervical specimen, urethral and rectal swabs also should be examined for gonococci in cases of assumed PID. Anaerobic bacteria regularly occur in the cervical flora of healthy women,[30] and their presence at this site in patients with PID is difficult to interpret with respect to the etiology of the PID. With regard to chlamydiae, some authors have recently stated that urethral cultures may be positive in up to 30% of women in whom the organism cannot be demonstrated in cervical specimens. In a recent study in which the culture results of urethral and cervical specimens were compared[120] such a discrepancy could not be demonstrated. On the contrary, cervical cultures were more often positive. This is in accordance with several earlier studies. The posterior vaginal fornix seems to be the preferred site for sampling for mycoplasmas from the lower genital tract. For the isolation of ureaplasmas, cervical and vaginal swabs have been shown to be equally efficient.[17]

Uterine cavity. As yet results of cultures from specimens collected from the uterine cavity in patients with acute PID have not been presented. For the collection of intrauterine specimens, Ledger and co-workers[43] recommended transabdominal endometrial aspiration. In PID patients such a study probably would provide important results because most agents causing this condition are believed to spread canalicularly via the uterine mucosa to the tubes (see following discussion). However, such a study must be questioned from an ethical point of view.

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*Hoorn, B.: Personal communication, 1974.

In cases of assumed genital tuberculosis in women in the fertile ages, menstrual blood should be collected for cultural studies.

Cul-de-sac and fallopian tubes. In cases of assumed salpingitis not subjected to laparoscopy or laparotomy, one must reckon with a comparatively high percentage of false diagnoses, viz., up to 25%, in clinically mild infections, as exemplified by Jacobson and Weström.[36] Furthermore, both these procedures afford the possibility of performing sampling direct from the cul-de-sac and/or the fallopian tubes. Biopsy specimens also may be obtained. Visual inspection of the pelvic organs also allows determination of the severity of the tubal inflammatory reaction, which seems to be of importance in the understanding of pathophysiologic processes in PID induced by various etiologic agents. Laparoscopy, as compared with laparotomy, involves less risk of microbial contamination of the specimens collected. During visual inspection of the tubes through the laparoscope, specimens may be obtained from the tubal lumina. Should the abdominal ostia be closed, a puncture can be made and material aspirated. The outcome of microbiologic studies of tubal specimens from patients with PID seems to depend on the experience of the surgeon who performs the sampling. For tubal sampling, a swab with a conical tip or an infant feeding catheter can be used. It seems essential that the sampling instrument be introduced into the lumen of the tube, and not just touching the fimbriated end. A difference in the outcome of analyses of these two types of specimens for chlamydiae might be equivalent to the discrepancy found in the frequency of isolation of these organisms between specimens collected from the urethral meatus and those collected by swabbing 3 to 4 cm up into the urethra.

As already discussed, in cases where laparotomy or laparoscopy is not performed, culdocentesis has been recommended as an alternative method for obtaining specimens from the cul-de-sac for etiologic studies. Comparison of the results of cultures of specimens collected from the cul-de-sac, at laparotomy, or in conjunction with laparoscopy vs. specimens obtained from the same patient by culdocentesis has revealed that often a greater number of species can be isolated from the latter type of specimen.[116] This indicates a risk of contamination of culdocentesis specimens by organisms of the vaginal or intestinal flora. Comparison between results of cultures of specimens from the cul-de-sac vs. the fallopian tubes in the same patient has shown that fewer species are recovered by culdocentesis than from fallopian tube specimens.[116] These observations indicate that tubal specimens are preferred in etiologic studies of AS (see also discussion of "*Neisseria gonorrhoeae*" and "Anaerobic infections").

Recent advances in laboratory diagnostic methods

Tests for tubal isoamylases. In the vast majority of cases of AS the level of tubal isoamylases in peritoneal fluid is reduced markedly.[136] In patients with assumed salpingitis in whom, for one reason or another, the tubes cannot be inspected visually, the levels of such amylases in a specimen collected by culdocentesis may be determined and compared to those in serum of the patient. The determination of the proportion of amylases in these specimens was found to have a higher diagnostic significance than the determination of acute-phase reactants in serum, the erythrocyte sedimentation rate, and white blood cell count in fluid from the cul-de-sac.[135]

***Neisseria gonorrhoeae*.** The development of transport media[28] for specimens to be studied for the presence of gonococci has done much to improve the quality of the diagnosis of gonococcal infections. However, when such specimens can be inoculated "bedside" and the culture plates incubated immediately thereafter, a higher isolation frequency has been demonstrated. Commercially available, ready-to-use media which after incubation can be sent to the laboratory for confirmation tests serve a similar purpose.

In a study in which gonococcal media based on various commercial dehydrated gonococcal agar bases (and prepared as recommended by the manufacturers) were compared with a newly developed variant of such media, the new medium was found to be superior with regard to the frequency of isolating *N. gonorrhoeae* (from urethral, cervical, and rectal specimens).[53] Other recently modified gonococcal media are those of Sng and colleagues[103] and Young.[139]

Recently, Soltesz and Mårdh[106] described an enrichment broth for *Neisseria gonorrhoeae* which, with isolated exceptions, allowed growth of all tested isolates of this organism. The broth medium, which may be given selective properties, is inexpensive and easy to prepare and does not contain blood products, apart from hemin. A medium with these properties has been lacking up to now.

Recently methods have been devised for the rapid identification of *N. gonorrhoeae*. One of them, the "coagglutination" test, utilizes the property of protein A to bind IgG antibodies nonspecifically (except for IgG3). By using *Staphylococcus aureus*, producing protein A and coated with gonococcal antibodies, gonococci may be agglutinated within a couple of minutes.[18] However, when serum-containing gonococcal media are used to isolate *N. gonorrhoeae*, difficulties may arise in interpreting the test result. Amato and associates[2] reported a method that avoids any need of fermentation tests and provides an answer within 4 hours. This method, which also eliminates the need to subculture suspect gonococcal colonies from the primary isolation plates, utilizes differences in the enzymatic profiles of *Neisseria gonorrhoeae* and other *Neisseria* species. Radiometric methods also have been reported as an alternative for identification of *Neisseria* species.[111] The use of head-space gas chromatography (discussion to follow) is another approach to the rapid identification of *N. gonorrhoeae*, for which less than 8 minutes are needed for each analysis. Schaffer and co-authors[99] recently used wheat germ lectin in agglutination tests for the differential diagnosis of *N. gonorrhoeae*.

At present, methods adapted for large-scale use in the routine clinical microbiologic laboratory are lacking for auxotyping or differentiation of *N. gonorrhoeae* by immunologic means.

In a comparison of four techniques for the detection of serum antibodies to *N. gonorrhoeae*, two gonococcal pili antibody tests, viz., an RIA[9] and an IHA,[91] were found to be highly and equally sensitive and specific. Although it is highly sensitive, the gonococcal complement fixation test, which has been used widely, has too low a specificity to be suitable for the study of deep-sited gonococcal infections such as AS.[50]

Anaerobic bacteria. For the diagnosis of anaerobic bacteria, the methods developed at Virginia Polytechnic Institute, Blacksburg,[32] and elsewhere have had a significant impact on diagnostic standards regarding anaerobic infections.

The application of gas chromatography to the identification of anaerobic bacteria enhances diagnostic rapidity. A further development within this field is the use of head-space gas chromatography for the same purpose.[42] This latter technique, which does not require derivation or extraction procedures, is extremely simple to perform and provides more information of differential diagnostic value than earlier used gonococcal methods. Analyses can be made on a 24 hour basis when using an automatic injector unit and connecting the gas chromatograph to a computer unit, thus providing an objective evaluation of the chromatograms obtained. Thus the organism in question may be identified without the presence of any personnel.

In addition to culturing, specimens collected from the cul-de-sac or the fallopian tubes of salpingitis patients should be subjected to staining procedures. Bacteria not detected by culture studies but found in stained smears of such specimens from untreated patients signifies inadequate culture techniques. Smears of such material should be stained shortly after sampling; otherwise, certain anaerobes may lose their ability to absorb stain. Acridine orange staining[40] eases the detection of bacteria in clinical specimens by providing a differential staining between bacteria and eukaryotic cells. Smears also may be prepared for immunofluorescence studies. Immunofluorescence test kits for the *Bacteroides fragilis* and *Bacteroides melaninogenicus* groups of bacteria are now available commercially. Groups headed by Holland[33] and Holst[34] found these test kits to have an acceptable sensitivity and specificity with regard to the two species mentioned. Clinical specimens also may be analyzed directly by gas chromatography for volatile fatty acids, which provides a rapid means for the detection of anaerobic infections, viz., in pelvic abscesses.[29]

***Mycoplasma hominis* and *Ureaplasma urealyticum*.** The use of swabs on wooden applicator sticks may prevent color change in urease tests on ureaplasmas, with subsequent false negative culture results, if solid media are used. Use of calcium alginate swabs also may result in a poor recovery of ureaplasmas. A rayon-tipped swab (Cepti-Seal Culturette, Marion Scientific Co.) produced the best culture results.[88] Similar studies on *M. hominis* do not appear to have been undertaken yet.

Recent evaluation of isolation techniques for genital mycoplasmas and ureaplasmas has shown that the quickest and best culture results are obtained when a biphasic medium is used. The sampling swab is kept for 1 day at 37° C in a broth medium, viz., B-medium for mycoplasmas and SU-medium for ureaplasmas.[70] The incubated media are then poured over the same type of media, but solidified with agar. The plates are incubated overnight at 37° C, whereafter the liquid phase is discharged. Colonies of *M. hominis* and *U. urealyticum* usually will emerge after another 1 to 2 days of incubation. When directly pouring plates with liquid transportation medium, colonies of mycoplasmas and ureaplasmas will emerge later, and the recovery frequency will be lower.

The mere use of the two types of medium mentioned generally will distinguish between *M. hominis* and *U. urealyticum*, which are almost exclusively those species of the family *Mycoplasmataceae* that can be recovered from genital tract specimens. In case of uncertainty as to the identity of the isolated organisms, immunofluorescence tests, as modified by Black and Krogsgaard-Jensen,[5] can be performed. Growth inhibition tests also might be used. In a recent modification of this latter test, antisera are applied directly to the surface of the culture plate, which is concave.[68]

Comparative studies in man and experimentally infected monkeys have shown that an IHA seems to be the most suitable technique for the determination of serum antibodies to *M. hominis*,[39, 69] Two antibody tests, one mycoplasmacidal, the other ureaplasmacidal, have been described also.[45]

Growth inhibition tests, as modified by Møller,[68] also can be used for the determination of serum antibodies to *M. hominis*, although this test is somewhat less sensitive than the IHA. The growth inhibition test has the advantage of being far easier to perform than other serologic methods described so far.

Chlamydia trachomatis. For the transportation of specimens to be cultured for *C. trachomatis*, a sucrose buffer (2-SP) containing antimicrobials usually is employed. For sampling, calcium alginate swabs often have been recommended. However, in studies in which such swabs were placed in experimentally infected 2-SP, a marked reduction in the inclusion count of *C. trachomatis* was found in subsequent isolations on tissue culture cells. Cotton-tipped swabs on wooden applicator sticks had the same effect. Of the various swabs available, a cotton-tipped steel swab (Medical Wire & Co.) was found to be the least toxic. The use of glass tubes for transportation of clinical specimens also may affect the culture results negatively. Such tubes contain metal ions, e.g., Br, Ca, Cl, Cu, K, Ni, Pb, and Zn, which can contribute to this effect.[64]

The diagnosis of *C. trachomatis* is based on the ability of the organism to produce characteristic intracytoplasmic inclusions in tissue culture cells. McCoy and HeLa 229 are the cell lines that have been employed most commonly for the isolation of *Chlamydia*. Treatment of HeLa cells with diethylaminoethylcellulose and pretreatment of McCoy cells with antimetabolites such as 5-iodo-2-deoxyuridine[132] and cytochalasin B[107] will render the cells more susceptible to infection with chlamydiae, thus causing genital infections. These methods were offered as alternatives to irradiation of the cells. In 1977, Ripa and Mårdh[93] recommended cycloheximide as another metabolite with which to treat McCoy cells to be used for the isolation of chlamydiae. Cycloheximide is added to the cultures at the time of specimen inoculation, which is advantageous. In comparative experimental studies using McCoy cells, the cycloheximide method was found to be more sensitive than techniques used earlier for the isolation of *C. trachomatis*.[23] For rapid detection of chlamydiae in cycloheximide-treated McCoy cells, Thomas and associates[121] used immunofluorescence staining of the cells. Salari and Ward[96] used fluorescent deoxyribonucleic acid–binding dyes for the same purpose. For the serologic diagnosis of genital chlamydial infections, the micro-IF test introduced by Wang and Graystone[128] is the most suitable technique described so far. In this technique, antibodies also can be determined by Ig class using isothiofluorescein-conjugated antihuman globulins. Complement fixation tests, which can detect antibodies to genus-specific antigens of *Chlamydia*, do not possess an acceptable sensitivity and specificity, with the possible exception of lymphogranuloma venereum infections.

In order to simplify the laborious procedure of the micro-IF test, originally described for immunotyping of chlamydial strains, the use of pools of antigens of the various immunotypes of *C. trachomatis* has been recommended.[125, 129] The use of pooled antigens somewhat reduces the sensitivity of the micro-IF test, but on the other hand, this modification makes it realistic to perform tests for chlamydial antibodies in routine diagnostic work. The use of formalinized antigens may simplify the handling of such antigens.[130]

The use of a single, broadly reacting antigen, viz., of immunotype LG 2, has been suggested also.[122] The use of such individual, easily grown antigens has been claimed by others to result in an unacceptable decrease in sensitivity and specificity.

The wearisome procedure of preparing antigens for micro-IF tests from infected yolk sac material or HeLa cell cultures stimulated the application of immunofluorescence antibody tests using tissue culture cells with inclusions of *C. trachomatis*, immunotypes E[92] and LGV 1,[3] as antigen. Techniques other than the micro-IF test also have been developed for serologic studies of chlamydial infections, e.g., an enzyme-linked immunosorbent assay,[44] hemolysis-in-gel test,[48] and a solid-phase radioimmunoassay,[118] but these tests have not been used widely so far.

Mycobacteria. The diagnosis of mycobacteria is laborious and time-consuming. The recent application of gas chromatography for their identification will increase diagnostic speed.[41, 124]

The direct demonstration of compounds characteristic of mycobacteria, viz., tuberculostearic and C_{32} mycocerosic acids, in clinical specimens is a specific and rapid means for the diagnosis of genital tuberculosis.[82] So far C_{32} mycocerosic acid has been demonstrated only in *Mycobacterium africanum, M. bovis, M. kansasii,* and *M. tuberculosis*.

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Experimental chlamydial salpingitis in the guinea pig

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Acute self-limited salpingitis developed when guinea pigs' fallopian tubes were infected with the guinea pig inclusion conjunctivitis agent *(Chlamydia psittaci)*. The disease peaked at approximately 8 days post infection and had diminished markedly by day 14. The inflammatory reaction was concentrated at the luminal surface. Chlamydial replication was demonstrated and inclusions were found in epithelial cells. (AM. J. OBSTET. GYNECOL. 138:952, 1980.)

ONE OF THE most common sexually transmitted human pathogens is *Chlamydia trachomatis*.[1] In Sweden, the organism has been recovered from the fallopian tubes of 30% of women with acute salpingitis.[2] Similar rates have not yet been reported in the United States where 0% to 10% of peritoneal specimens have yielded the organism, but serologic evidence has suggested that *Chlamydia* may be involved in approximately 20% of acute salpingitis cases in this country.[3–5] Thus, development of an animal model would help investigations of the pathogenesis of this disease. Ripa and associates[6] have shown that chlamydiae can infect the fallopian tubes of grivet monkeys and salpingitis will develop, but studies in this experimental host are both difficult and very expensive to perform.

Hutchinson and colleagues[7] have shown that human fallopian tubes cultured in vitro are capable of supporting replication of *C. trachomatis*, but there appears to be little observable damage to the tubes. We have confirmed this observation by demonstrating considerable chlamydial replication ($>10^3$ to 14^4 inclusion forming units). However, even when we held cultures for several weeks post infection (persistence of the organism was shown and inclusion could be seen in sections) we observed no microscopic change in ciliary activity and no microscopic destruction of the architecture of the tubes.

The naturally occurring *Chlamydia psittaci* infection of guinea pigs, guinea pig inclusion conjunctivitis (GPIC), suggested the use of the guinea pig fallopian tube. This disease, first described by Murray,[8] who later extensively studied the immune response, is apparently an infection of the female genital tract in guinea pigs which is transmitted to the newborn animal.

The young guinea pig develops an acute self-limited conjunctivitis and is thereafter relatively resistant to ocular reinfection. Experimental studies have shown that the genital tract of the guinea pig is capable of supporting the infection and that the guinea pigs born while the birth canal is experimentally infected will acquire the infection.[9, 10] Rectal infection also can be induced experimentally.[11] White and colleagues[12] have shown that immunosuppression of guinea pigs with vaginal infection prevents the normal resolution of the disease and the infection ascends into the fallopian tubes, resulting in salpingitis. We attempted to induce salpingitis in normal guinea pigs to establish a model for further studies of chlamydial infection in the fallopian tube.

It appears from studies of the human fallopian tube that chlamydial salpingitis cannot be studied readily in vitro because the host's inflammatory response seems to make a vital contribution to the disease process. By establishing a model in the guinea pig, it would be possible to compare infection in guinea pig fallopian tubes maintained in organ culture with in vivo infection of

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This work was supported in part by Public Health Service grants HD-12271 and EY-1205 from the National Institutes of Health.

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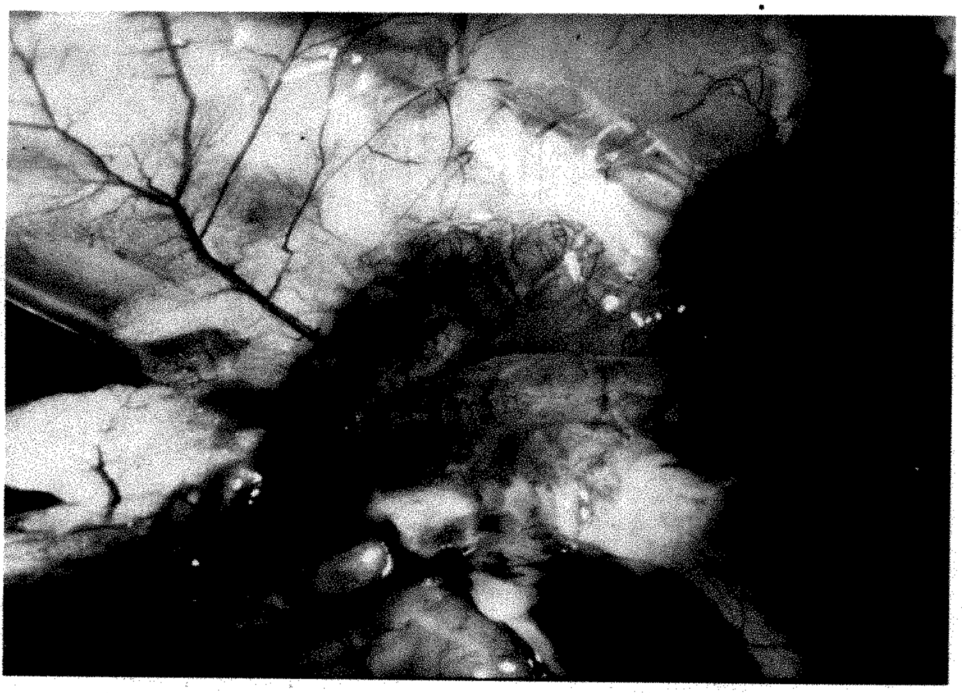

Fig. 1. Seven days post infection. The fallopian tube is swollen and hyperemic. It presents a "scalloped" appearance as it leaves the uterine horn and passes over the top of the ovary. The bottom of the ovary is coated with pus.

the falopian tubes. We report preliminary observation on the effects of GPIC agent on the fallopian tube in vivo.

Materials and methods

Chlamydia-free female guinea pigs were obtained from Simonsen Laboratories in California and from Charles River in Massachusetts. The animals were anesthetized with ketamine (44 mg/kg) and acepromazine (2 mg/kg) administered in combination. A local anesthetic (1% lidocaine) was infiltrated into the abdominal walls and the uterine horns were exposed by laparotomy. The proximal uterine horn was manually compressed and 0.5 ml inoculum was introduced distally with a 24 gauge needle. This volume was chosen because preliminary experiments showed that 0.4 to 0.5 ml was required for the inoculum to reach the fimbria. The surgical incision was closed by surgical skin clips. In different experiments the animals received varying amounts of agent and were killed at day 8 or at intervals from 2 to 21 days after receiving standard inocula (10^4 LD_{50}). In each experiment, control animals underwent the same procedures but were inoculated with uninfected tissue culture supernatant. Upon sacrifice the animals were bled to determine antichlamydial antibody titers and the fallopian tubes were examined microscopically. Portions of the fallopian tubes were dissected out and placed in Bouin's fixative for histopathologic examination. Small portions also were dissected out and frozen at −70° C for determination of infectiousness.

Microbiologic studies

Chlamydial titrations were performed in cycloheximide-treated LLC-MK2 cells (which we have found to be highly susceptible to GPIC agent) and in the yolk sac of embryonated hens' eggs.[13] Infective inocula were prepared in tissue culture. The inoculum was cultured for 72 hours in McCoy cells, suspended by shaking cells with glass beads, and centrifuged at 200 × g to separate cell debris. Dilutions were performed in tissue culture medium (minimal essential medium with 10% fetal calf serum). Antibodies to chlamydiae were determined by the microimmunofluorescence test as described by Wang and Grayston,[14] but using GPIC agent as antigen. The GPIC agent used in these studies was isolated in this laboratory.[15]

Results

Clinical course and gross observations. The inoculated animals did not become febrile nor did they appear ill. The following observations were made from animals killed at 1 to 2 day intervals. With slight variation for infecting inoculum, salpingitis was first appar-

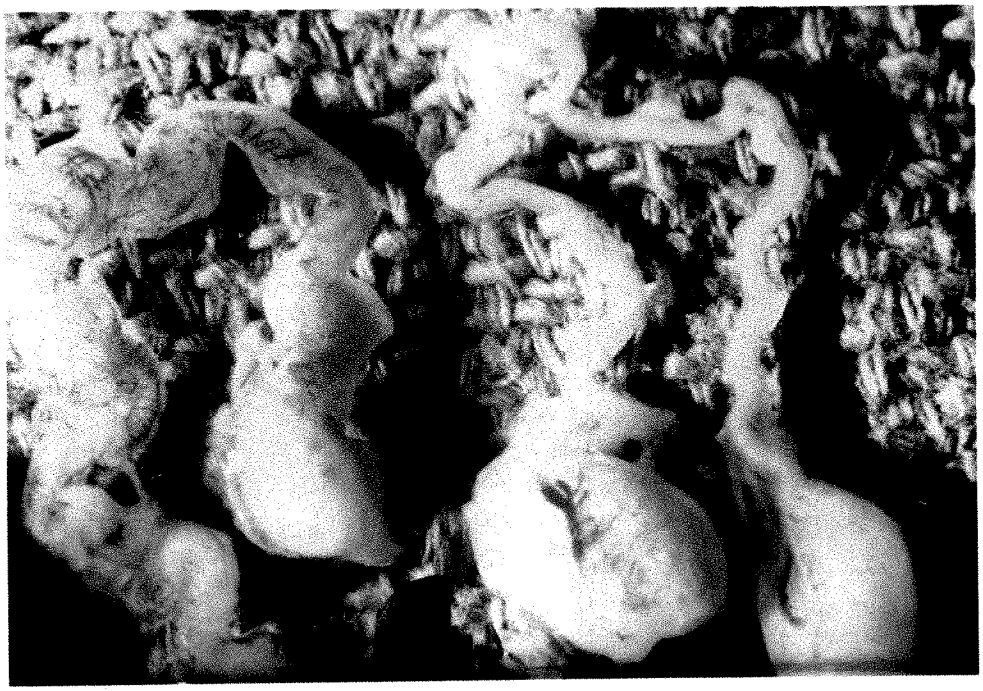

Fig. 2. On the left is a swollen and highly vascularized fallopian tube (7 days post infection). On the right is a normal fallopian tube.

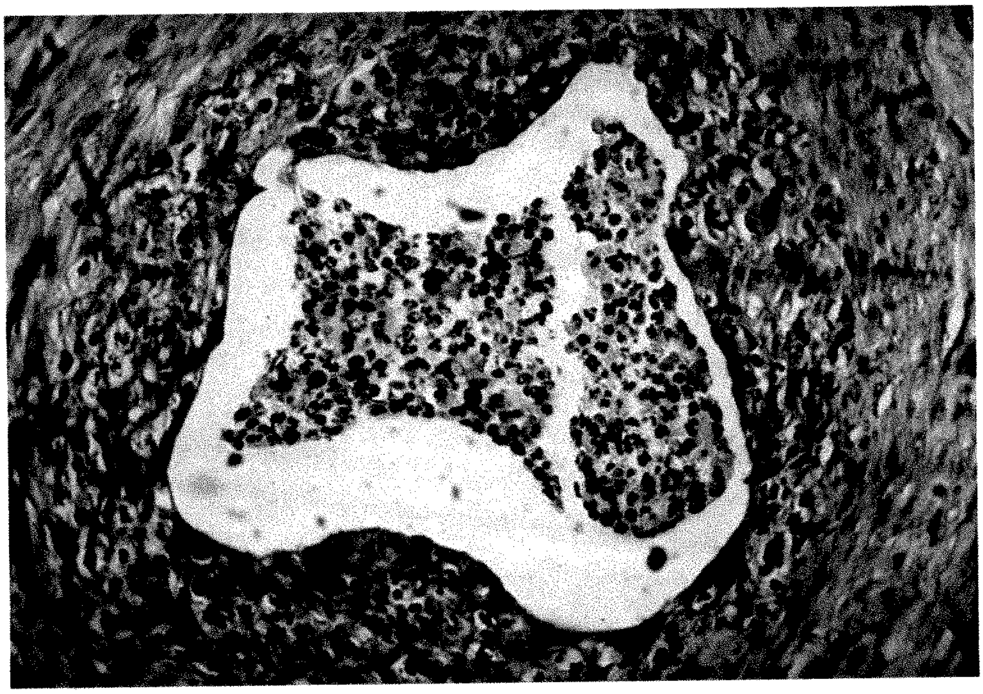

Fig. 3. Marked inflammatory reaction 8 days post infection is largely restricted to the luminal aspects of the fallopian tube and extends into the epithelial surface. (Giemsa stain. Magnification ×250.)

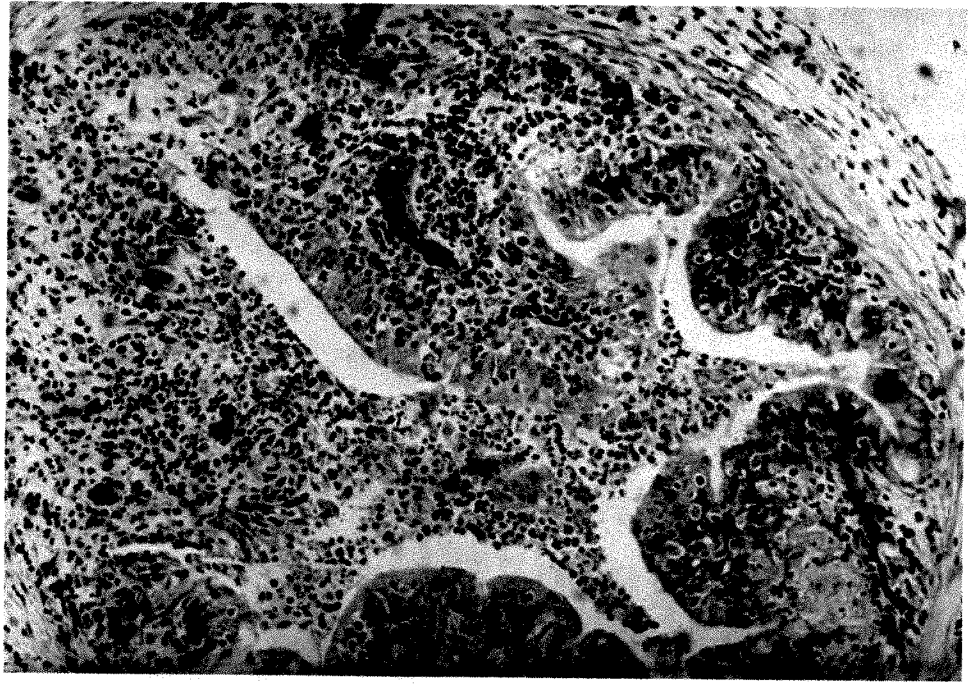

Fig. 4. Eight days post infection. Lumen of fallopian tube is filled with acute inflammatory infiltrate. There are scattered areas of epithelial cell necrosis and sloughing. There are stromal edema and a transmural mononuclear infiltrate. (Giemsa stain. Magnification × 160.)

ent approximately 4 days post inoculation (animals receiving $\geq 10^4$ Egg LD_{50} showed inflammation at 2 days). The disease followed an acute course, peaking in intensity at approximately 8 to 10 days post inoculation. At the peak of reaction the fallopian tubes were edematous, distended, and hyperemic and the vascular pattern of the serosa became very apparent (Figs. 1 and 2). The purulent discharge at the fimbria often covered the ovary. Obvious signs of inflammation had disappeared by 14 days post inoculation but adhesions had developed between the ovary–fallopian tube and adjacent organs.

Histologic findings. The acute inflammatory reaction was largely restricted to the luminal aspect of the fallopian tube and involved the epithelial surfaces (Fig. 3). The lumen became filled with purulent material and necrotic debris. There was a marked inflammatory reaction initially of polymorphonuclear cells and later (>7 days) of mixed cell type that extended through the epithelium into the stroma. Stromal edema was marked. The tubal fimbria were blunted and expanded by a mononuclear cell infiltrate that extended into the submucosa. The inflammatory reaction peaked between 6 to 8 days post infection. Inclusions were seen only in epithelial cells lining the lumen. None was found in the stroma or serosa.

The general architecture of the fallopian tube usually was maintained, but a few sections showed obvious destruction of the epithelium (Fig. 4). Here there was also a transmural mononuclear infiltrate (lymphocytes, histiocytes, and scattered plasma cells) that extended from the lamina propria through the serosa. By day 14 the lumen had cleared and inflammatory cells were sparse. Most of the epithelium had returned to normal.

Table I. Laboratory results on fallopian tubes 8 days after infection with GPIC agent

Infecting inoculum	*Salpingitis*	*Chlamydia recovery*	*Antibody level*	
			IgG	*IgM*
$10^{4.3}$*	6/6	$10^{4.2}$*	128†	64
$10^{3.3}$	6/6	$10^{3.2}$	43	21
$10^{2.3}$	6/6	$10^{3.2}$	16	8
$10^{1.3}$	3/3	$10^{3.0}$	—	—
$10^{0.3}$	0/3	—‡	—	—
0	0/6	—	—	—

*Egg LD_{50} inoculated or per gram of tissue sample.

†Geometric mean titer.

‡Negative.

Animals inoculated with tissue culture medium showed no signs of inflammation.

Microbiologic results. The fallopian tubes were quite susceptible to the chlamydial infection. A titration showed that only 20 Egg LD_{50} were required to cause salpingitis (Table I). Chlamydial replication in the fallopian tubes could be demonstrated both by inclusions found in sections and by recovery of higher titers of the organism than had been inoculated for those animals that had received less than 10^3 LD_{50} (Table I). The organism could be recovered from some animals for at least 14 days post inoculation.

Antibody titers $>1:8$ were not detected until 7 days post infection. At 8 days post inoculation the antibody titers appeared to be directly related to the infecting inocula (Table I). IgG titers usually were higher and were detected at the same time as IgM responses. Both seemed to peak at approximately 21 days post infection when titers of $\geq 1:1{,}024$ were observed.

Discussion

The guinea pigs that received GPIC agent inoculated directly into the uterine horn developed an acute self-limited salpingitis.

The chlamydiae replicated within the epithelial cells lining the lumen and the disease process seemed to be restricted largely to that site. The active inflammatory process peaked 7 to 10 days post infection, with considerable resolution by 14 days. This time sequence is quite similar to the course of the conjunctivitis produced by this agent.

This experimental salpingitis model may simulate the evolution of human chlamydial salpingitis. It is likely that many cases of salpingitis in women are also self-limited and it may be that more tubal damage results from repeated attacks. It is our intention to further exploit this guinea pig model and to discover the effects of repeated infection with *Chlamydia* and what happens when chlamydiae are introduced in combination with (or followed by) other pathogens.

We did not observe any grossly apparent sequelae other than adhesions. Small areas of damaged epithelium could be missed easily by incomplete sampling. We have not examined animals any later than 21 days post infection for sequelae that may be identified later. However, it is obvious that the ultimate test of fallopian tube function is uterine pregnancy. We intend to expose animals to multiple infections with chlamydia and other pathogens and will then attempt to breed them. This may be a better indicator of tubal damage than histopathologic demonstration of lesions. The role of immunity in restricting the infective process or in enhancing the disease process will be investigated also. The guinea pig model may prove inappropriate for this aspect as a model for human disease because resistance to reinfection in the guinea pig may be more solid than in man.

We intend to study the effect of the GPIC agent on guinea pig fallopian tubes maintained in vitro. By comparing the responses seen in organ cultures vs. those seen in the intact animal, we may be able to extrapolate from the responses seen with human fallopian tubes maintained in vitro and infected with *Chlamydia trachomatis* to the pathologic agents involved in human salpingitis with *C. trachomatis*.

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Chlamydia trachomatis in acute salpingitis

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Of the 228 hospitalized patients who had acute salpingitis, 69 (30%) harbored *Chlamydia trachomatis* in the lower genital tract (LGT). The mean antichlamydial IgG immunofluorescence antibody titer was 264 in sera from the *C. trachomatis* culture–positive patients but only 49 in culture-negative patients. In paired sera obtained from 167 of the patients, 32% of the culture-positive patients and 13% of the culture-negative patients showed significant titer change. Patients with abscess formation had higher mean antichlamydial antibody titer than the group with no abscess (192 vs. 53), although there was no difference between the groups in positive chlamydial cultures from the LGT (33% vs. 29%). Overall, this study indicates that *C. trachomatis* is a common etiologic agent in acute salpingitis, emphasizing the need to treat chlamydial cervicitis before this complication develops. (AM. J. OBSTET. GYNECOL. 138:957, 1980.)

THE INCIDENCE of acute salpingitis (synonym, pelvic inflammatory disease), one of the most common gynecologic disorders, seems to be on the increase.[1] It affects mostly young women, and the late sequelae are often severe: involuntary childlessness, increased risk of ectopic pregnancy, and chronic abdominal pain.[2] Acute salpingitis has classically been divided into gonococcal and nongonococcal forms on the basis of recovery or nonrecovery of *Neisseria gonorrhoeae* from the lower genital tract. In many western societies, however, the commonest cause of sexually transmitted diseases is no longer *N. gonorrhoeae* but *Chlamydia trachomatis*.[3, 4] This prospective study adds to the earlier evidence that *C. trachomatis* is involved frequently in acute salpingitis.

Patients and methods

Patients. The study population consisted of 228 consecutively examined women with acute salpingitis not associated with surgery or instrumentation who presented in the emergency room of I-II Depart-ments of Obstetrics and Gynecology, University Central Hospital, Helsinki, between October, 1977, and September, 1979. All were admitted to the hospital because of severe symptoms. The clinical diagnosis of acute salpingitis was based on common criteria,[5] which included lower abdominal pain, abnormal vaginal discharge, adnexal tenderness, erythrocyte sedimentation rate ≥15 in the first hour, and usually fever (≥38° C). Two different groups of salpingitis were considered on the basis of abscess formation (defined as swellings, tender to palpation, located beside the uterus which were either unilateral or bilateral).

Microbiologic and serologic methods. Cervical and urethral specimens for culture of *N. gonorrhoeae* and *C. trachomatis* were collected at the time of admission and handled as described elsewhere.[6] Chlamydial isolations were made in irradiated McCoy cells.

Acute-phase sera were obtained from all patients. Both acute-phase and convalescent-phase sera were obtained (at intervals of 1 to 3 weeks) from 167 patients. The sera were stored at −20° C until assayed. IgG antibodies to *C. trachomatis* were determined by an immunofluorescence antibody test (IFAT) in which inclusions of *C. trachomatis* serotype L2 (434Bu) in cell culture served as antigen.[7] The criterion for positive findings in this assay was either a significant (≥fourfold) change in antibody concentration or a value of ≥512, which is above the ninety-ninth percentile seen in a series of serum specimens from healthy Finnish adults.[8]

Statistical significance was evaluated with the chi-square test and Student's t test.

Results

The mean age of the patients was 27.8 years (range, 15 to 49), and 149 (65%) of them were less than 30 years of age. Eighty-two (36%) were nulliparous; 114 (50%) were fitted with an intrauterine contraceptive device and 25 (11%) were taking oral contraceptives. The gynecologic examination revealed acute salpingitis with abscess formation in 86 (38%) cases and acute salpingitis without pelvic mass in 142 (62%) cases.

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Table I. Results of cultures for *C. trachomatis* and *N. gonorrhoeae* from the lower genital tract of 228 patients with acute salpingitis

Culture for N. gonorrhoeae	*Culture for* C. trachomatis		
	Positive	*Negative*	*Total*
Positive	17	43	60
Negative	52	116	168
Total	69	159	228

Table II. Age distribution and percent *C. trachomatis* culture–positive results in 228 patients with acute salpingitis

Age group (yr)	C. trachomatis *cultures (no. positive/total)*
<20	12/20 (60%*)
20-29	41/129 (32%)
30-39	14/60 (23%)
≥40	2/19 (15%)

*Percent positive.

Lower genital tract (LGT) culture for *C. trachomatis* was positive in 69 (30%) patients (in 33 cases from both the cervix and urethra, in 26 cases only from the cervix, and in 10 cases only from the urethra), and culture for *N. gonorrhoeae* was positive in 60 (26%) patients (Table I). Of the *C. trachomatis* culture–postive and culture–negative patients, 17 (25%) and 43 (27%), respectively, also had gonorrhea. The rate of *C. trachomatis* culture positivity was highest (60%) in the youngest age group (<20 years) as compared to any other age group (Table II).

The distribution of antichlamydial IgG antibody titers is shown in Table III. In total, 56 (25%) patients had exceptionally high antibody titers of ≥512. Such a high titer occurred in 28 of 69 (41%) *C. trachomatis* culture–positive patients and in 28 of 159 (18%) culture–negative patients ($p < 0.01$). The mean antibody titers in the two groups were 264 and 49, respectively.

Among 167 patients from whom paired sera were collected, significant titer changes were observed in 17 of 54 (32%) *C. trachomatis* culture–positive patients and in 15 of 113 (13%) culture–negative patients ($p < 0.01$).

The patients with abscess formation had higher mean anti–*C. trachomatis* antibody titer than the group with no abscess (192 vs. 53, $p < 0.001$). Besides anti–*C. trachomatis* antibody level, abscess formation also correlated with a longer mean duration of symptoms (11.0 vs. 4.7 days, $p < 0.001$). By contrast, there was no correlation between positive LGT culture for *C. trachomatis* and abscess formation (Table IV).

Discussion

Nontuberculous salpingitis has classically been divided into gonococcal and nongonococcal forms depending on the isolation of gonococci from the LGT. However, it is unreasonable today to attribute most cases of salpingitis to the gonococcus, at least in populations where the incidence of gonorrhea is declining.[1] In a large proportion of patients with salpingitis (and their sexual partners) *N. gonorrhoeae* cannot be isolated from the LGT. This has raised questions about the etiology and pathogenesis of nongonococcal salpingitis in particular. Earlier studies have shown that with the exception of *N. gonorrhoeae* no difference exists in the cervical bacterial cultures from patients with gonococcal and those with nongonococcal salpingitis.[5]

This study provides support to the concept that *C. trachomatis* often is involved in acute salpingitis. In total, 30% of the patients harbored *C. trachomatis* in the LGT and 24% had exceptionally high (≥512) anti–*C. trachomatis* immunofluorescence antibody titers in the sera. Such a high titer occurred significantly more often in the sera of *C. trachomatis* culture-positive patients. The fact that 18% of the culture-negative patients also had such high antibody levels needs explaining. Technical failure in isolation as well as recent treatment with antimicrobial agents might have been responsible for the negative culture result in some cases. This was consistent with the finding that 13% of the 113 patients from whom paired sera were obtained but who were culture-negative for *C. trachomatis* showed significant titer change. In some cases, high titers also may be due to a past chlamydial infection since relatively high levels of antibody probably persist for a long time after chlamydiae have been cleared. This, however, probably was not very often the case since positive serology did not correlate with previous history of salpingitis.[9]

One might postulate that *C. trachomatis* behaves in the same manner as *N. gonorrhoeae* and has an important role in initiating the infection by damaging the mucosal surfaces. As a result, cervical opportunistic bacteria may gain access to the upper genital tract. Studies utilizing culdocentesis and the modern techniques of anaerobic microbiology have suggested that obligate anaerobes are associated with a varying proportion of acute salpingitis cases. Whether these are pathogenic or superinfections is not clear. Culdocentesis very often yields mixed cultures, the value of which is difficult to interpret because of possible contamination by the complex normal flora of the vagina. Recent serologic studies, however, support the frequently polymicrobial

Table III. Distribution of antichlamydial IgG IFAT titers of 228 patients with acute salpingitis

Culture for C. trachomatis	No.	IFAT titer											Mean titer*	No. ≥512
		<8	8	16	32	64	128	256	512	1,024	2,048	4,096		
Positive	69	0	0	2	4	11	11	13	8	13	6	1	264†	28‡
Negative	159	32	17	5	13	14	34	16	12	11	4	1	49†	28‡
Total	228	32	17	7	17	25	45	29	20	24	10	2	82	56

*The value 2 was given to the titers of <8.
†$p < 0.001$.
‡$p < 0.01$.

nature of acute salpingitis in which, in addition to *N. gonorrhoeae* and *C. trachomatis*, enteric bacteria and encapsulated *Bacteroides fragilis* play an important role.[9, 10]

The patients who had more severe disease with abscess formation showed a significantly higher anti–*C. trachomatis* antibody level than the group with no abscess, although there was no correlation between the isolation of *C. trachomatis* and the severity of the disease. Similarly, previous serologic studies[11, 12] in patients with acute salpingitis have shown a correlation between the severity of the salpingitis and the magnitude of the antichlamydial antibody response. In the present study, this finding also was consistent with the fact that the patients with abscess formation had longer mean duration of symptoms, thus offering more time for antibody to develop.

This study indicates that *C. trachomatis* is a common etiologic agent in acute salpingitis and emphasizes the need to treat chlamydial cervicitis before this complication develops. Diagnostic facilities for *C. trachomatis* should be available in any major medical center for diagnosing and treating patients with salpingitis. Such facilities should also be available for diagnosis and treatment in those women who attend the clinics for reasons other than acute salpingitis, many of whom would go untreated and therefore would be at increased risk for acute salpingitis if screening facilities were not available.

Table IV. *C. trachomatis* culture positivity and mean antichlamydial IFAT titers, compared with the clinical finding and the duration of pelvic symptoms

Clinical finding	Duration of symptoms (days ± SD)	C. trachomatis isolated	Mean titer
No abscess	4.7 ± 3.3*	41/142 (29%)	53*
Abscess	11.0 ± 8.6*	28/86 (33%)	192*

*$p < 0.001$.

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Chlamydia trachomatis infection in patients with laparoscopically verified acute salpingitis

Results of isolation and antibody determinations

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Culture and serology studies have shown *Chlamydia trachomatis* (CT) to be one of the causes of acute salpingitis (AS). In the present investigation, results of cervical cultures were correlated with serum antibody titers to CT in patients with laparoscopically verified AS. Serum samples from 206 patients, including paired sera from 80, were assayed. Of 206 patients, 118 had chlamydial IgG antibody titers of 1:64 or more. Patients with negative cultures for CT and an IgG titer of 1:64 or more had a significantly higher geometric mean titer than corresponding patients with positive cultures. In paired sera, a seroconversion or a fourfold or greater rise in IgG titer to CT was demonstrated in 35%, while a further 11% had detectable IgM antibody in a titer of 1:8 or more. The overall isolation frequency of CT was 33%, compared with 19% for *Neisseria gonorrhoeae*. (AM. J. OBSTET. GYNECOL. 138:960, 1980.)

DURING THE PAST decade our knowledge of the clinical spectrum of infections caused by *Chlamydia trachomatis* has expanded. Apart from being the microbiologic cause of trachoma and related eye infections, *C. trachomatis* is recognized today as a major cause of nongonococcal urethritis in men and of lower genital tract infection in the women. The organism also is known to be an etiologic agent of acute salpingitis,[1] acute epididymitis,[2] and neonatal pneumonitis.[3]

The relative importance of *C. trachomatis* in acute salpingitis (AS) is a function of the prevalence of the organism in the population and of the prevalence of gonorrhea. In Sweden the incidence of gonococcal infections has decreased by 50% since 1970. The frequency of cervical gonorrhea in patients with AS in our hospital catchment region was 50% in 1970, and declined to 20% in 1976-78. The incidence of AS increased during the same period.[4]

In past studies we have shown that *C. trachomatis* can be isolated from the cervix and the fallopian tubes in approximately one third of patients with AS admitted to the University Hospital of Lund.[1] Of 143 such patients we showed a prevalence of IgG antibodies to *C. trachomatis* in a titer of ≥1:64 in 62%.[5] This investigation extends our previous findings concerning the frequency of antibodies to *C. trachomatis* in patients with laparoscopically verified AS and correlates them with the results of cervical cultures.

Patients and methods

Patients. Two hundred and six inpatients, 15 to 45 years of age (mean, 23.5; standard deviation, 5.7), with laparoscopically verified AS, treated at the Department of Obstetrics and Gynecology, University Hospital, Lund, during the years 1973-78, comprise the study population.

Diagnostic laparoscopy was performed on the day of admission in each case. The laparoscopic technique

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Table I. Distribution of IgG chlamydial antibody titers in sera from 206 women with acute salpingitis, 50 women with a LGTI, and 50 postpartum women (controls)

	No. of patients	*Reciprocal IgG chlamydial antibody titers*											*Patients with ≥1:64 (%)*	*GMT (patients with ≥1:16)*
		<16	*16*	*32*	*64*	*128*	*256*	*512*	*1,024*	*2,048*	*4,096*	*8,192*		
Grade of salpingitis														
Mild	74	28	6	11	12	8	6	2	—	1	—	—	39	72
Moderate	78	19	4	3	12	14	12	5	3	1	3	2	67	186
Severe	54	11	3	3	5	1	10	6	9	4	2	—	69	311
Total acute salpingitis	206	58	13	17	29	23	28	13	12	6	5	2	57	161
LGTI	50	31	5	6	4	2	2	—	—	—	—	—	16	44
Controls	50	45	2	1	—	2	—	—	—	—	—	—	4	42

Table II. Distribution of IgM chlamydial antibody titers in 206 women with acute salpingitis

Grade of salpingitis	*No. of patients*	*Reciprocal IgM antibody titers*								*Patients with ≥1:8 (%)*	*GMT (patients with ≥1:8)*
		<8	*8*	*16*	*32*	*64*	*128*	*256*	*512*		
Mild	74	65	4	3	1	1	—	—	—	12	15
Moderate	78	61	8	1	3	2	2	—	1	22	24
Severe	54	46	1	1	1	—	4	1	—	15	64
Totals	206	172	13	5	5	3	6	1	1	17	27

and the criteria for a laparoscopic diagnosis of AS have been described in detail elsewhere.[6] The inflammatory changes of the fallopian tubes, as seen at laparoscopy, were graded as mild (reddened serosa on the tubes, edema of the tubal wall, and purulent discharge from the abdominal orifice), moderate (more advanced inflammatory changes confined to the tubes, such as tubal swelling, closure of the abdominal orifices, and fibrin deposits on the serosal surfaces of the tubes), or severe (pelvic peritonitis or abscess formation).

Fifty women with a lower genital tract infection (LGTI) were used as controls. These women were admitted because of symptoms of AS but laparoscopic evidence of this disease was lacking. Sera from 50 nonselected women who had recently given birth were assayed for chlamydial antibodies also (controls).

Collection of specimens. Serum samples were collected on the day of admission from the 206 women with AS and the 50 women with LGTI. From 80 of the 206 AS patients an additional serum sample was drawn 4 to 21 days later. Sera from the 50 control women were taken just after delivery of their babies. The serum samples were frozen at −20° C until assay.

Specimens for culture of *C. trachomatis* were obtained by rotating a sterile, cotton-tipped swab in the cervical canal and transporting it to the laboratory in sucrose-phosphate buffer containing gentamicin, vancomycin, and amphotericin B. Specimens not cultured within 48 hours were frozen at −20° C until assay (within 1 week). Valid samples were obtained from 156 of the patients with AS. From all 206 AS patients, specimens for culture of *Neisseria gonorrhoeae* were obtained from the cervical canal, the urethral orifice, and the rectum, with a cotton-tipped swab treated with charcoal. Transport was in a modified Stuarts medium.

Table III. Results of cervical cultures for *Chlamydia trachomatis* and determination of serum chlamydial antibodies from 156 patients with acute salpingitis

Grade of salpingitis	*No. of patients*	C. trachomatis *isolated*		*Serologically positive**	
		n	*%*	*n*	*%*
Mild	58	20	34	23	40
Moderate	56	19	34	35	63
Severe	42	13	31	29	69
Totals	156	52	33	87	56

*Serologically positive = serum chlamydial IgG ≥ 1:64 and/or IgM ≥ 1:8

Isolation and serologic procedures. We isolated *C. trachomatis* on cycloheximide-treated McCoy cells.[7] *N. gonorrhoeae* was isolated on a modified McLeod medium and identified with sugar fermentation tests. IgG and IgM antibodies to *C. trachomatis* were determined using a modified microimmunofluorescence test.[3]

Statistical analysis. Statistical methods used were the chi-square test for testing frequencies and the analysis

Table IV. Correlation of results of cervical cultures for *Chlamydia trachomatis* with a titer of serum IgG chlamydial antibody ≥1:64 (seropositive) in 156 women with acute salpingitis

Grade of salpingitis	*No. of patients*	*Culture-neg./seroneg.*			*Culture-pos./seroneg.*			*Culture-pos./seropos.*			*Culture-neg./seropos.*		
		n	*%*	*GMT**	*n*	*%*	*GMT*	*n*	*%*	*GMT*	*n*	*%*	*GMT*
Mild	58	23	40	—	14	24	—	6	10	102	15	26	147
Moderate	56	16	29	—	5	9	—	14	25	100	21	38	345
Severe	42	13	31	—	1	2	—	12	29	362	16	38	558
Totals	156	52	33	—	20	13	—	32	21	162	52	33	313

*Geometric mean titer of serum IgG chlamydial antibody ≥1:64.

Table V. Presence of chlamydial antibody (IgG and IgM) in paired sera from 80 patients with acute salpingitis

	No. of patients	*%*
Seroconversion or ≥ fourfold increase in IgG titer	28*	35
IgM ≥ 1:8, IgG stable	9	11
IgG ≥ 1:64 (stable), no detectable IgM	24	30
IgG <1:64 (stable), no detectable IgM	19	24

*IgM ≥ 1:8 in eight of these patients.

of variance (one- and two-way analysis of variance). In cases where significant differences were found, the multiple test according to Duncan was used.

Results

Fifty-seven percent (118 of 206) of the patients with AS had chlamydial IgG antibody levels of ≥1:64 (Table I). The geometric mean titer (GMT) of antibody in the sera from patients with an IgG titer of ≥1:64 was 1:161. There was a statistically significant difference ($p < 0.0001$) between the grade of salpingitis and the GMT. Significantly more of the patients with moderate or severe vs. mild AS had IgG antibody levels ≥1:64 ($p = 0.0004$). For comparison, the results of IgG determinations in serum from 50 patients with LGTI and 50 control patients also are listed in Table I. The differences in frequency of occurrence of patients with IgG titer ≥1:64 were highly significant ($p < 0.0001$) as was the difference in GMT between AS patients vs. the LGTI patients and controls ($p = 0.0001$).

IgM antibodies to *C. trachomatis* could be detected only in serum from 17% (34 of 206) of the women with AS (Table II). The difference in GMT between the groups was statistically significant ($p = 0.04$).

Results of antibody determination and culture from the 156 AS patients from whom cervical cultures for *C. trachomatis* were taken are shown in Table III. Of these patients, 56% had a titer of IgM antibody of ≥1:8 and/or IgG antibody of ≥1:64, while 33% were culture-positive. There were no differences in isolation results between patients with various inflammatory grades of AS, whereas those with moderate and severe AS were seropositive significantly more often than the patients with mild AS ($p = 0.002$).

The relationship between cervical cultures for *C. trachomatis* and antibody determinations, as seen in Table III, can be elucidated further by dividing the patients into four groups (Table IV). Seropositivity is defined as a titer of chlamydial IgG antibody of *1:64*. Thirty-three percent of the patients had no laboratory evidence of a chlamydial infection, i.e., they were culture- and seronegative. Thirteen percent of all the patients had a positive cervical culture but were seronegative; most of these belonged to the group with mild AS. Twenty-one percent of the patients were both culture- and seropositive. Finally, 33% were culture-negative but seropositive. The GMT of antibody in this last group of patients was significantly higher than the GMT of the culture-positive group ($p = 0.004$).

The results of antibody determinations on sera from 80 patients with AS from whom paired sera were obtained are shown in Table V. A total of 46% had serologic evidence of an active chlamydial infection; that is, a seroconversion or fourfold rise in IgG titer occurred or else IgM was demonstrable in a titer of ≥1:8. IgM antibody in a titer of 1:8 was demonstrable in a total of 21% (17 of 80) of the patients. Twenty-four of the 80 (30%) had a titer of IgG antibody of ≥1:64 in their sera, yet no demonstrable titer change or IgM.

N. gonorrhoeae was isolated from 19% (39 of 206) of the AS patients (mild group, 15%; moderate group, 21%; severe group, 22%). There was no correlation between antibody titers to *C. trachomatis* and culture results for gonococci.

Discussion

Acute salpingitis usually is divided into two categories—gonococcal and nongonococcal. This classification is not based on the finding of gonococci in the fallopian tubes but on the isolation of *N. gonorrhoeae* from the cervix, whereas other microbes, without concomitant gonococci, have been found in cul-de-sac fluid from patients with endocervical gonorrhea, few patients have evidence of gonococcal infection without a simultaneous positive culture from the cervix.[9]

The frequency of cervical *C. trachomatis* infection and gonorrhea in AS patients is a function of the prevalence of these microbes in the population. In the study area in southern Sweden, 19% of the patients with AS were found to have cervical gonorrhea during the study period (1973-78). *C. trachomatis* was isolated from the cervix in 33% of the patients.

Fifty-seven percent of the AS patients had an IgG titer of chlamydial antibodies of $\geq 1:64$, a titer earlier suggested as being indicative of an active or recent chlamydial infection.[5] However, it is not suggested that this antibody level is a suitable titer limit capable of use for diagnosis in the individual case. In a previous report we found that 64% of 143 AS patients had IgG antibodies to *C. trachomatis* at this level,[5] a figure agreeing fairly closely with the 56% found by Paavonen and associates[10] and the 59% found by Simmons and colleagues.[11] Paavonen and his group reported 26% of the patients in the former study to be isolation-positive for chlamydiae.

In our study and in that of Paavonen and co-workers AS patients who had serum IgG antibody to *C. trachomatis* in a titer of $\geq 1:64$ numbered twice those who were isolation-positive. This discrepancy could be due to a lack of sensitivity in culture techniques used, or perhaps the antibodies found could be trace remnants of previous chlamydial infections.

The 156 patients with AS from whom valid cultures were taken could be separated into four groups according to the culture and serum results using the aforementioned IgG titer to indicate seropositivity. One third of these patients were culture-negative yet seropositive and had the highest geometric mean titers, the difference between the culture- and seropositive groups being significant ($p = 0.0004$) (Table IV).

Thirty-seven of 80 patients (46%) from whom paired sera were available had serologic signs of active infection, i.e., seroconversion, fourfold or greater increase in IgG titer, and/or presence of IgM to chlamydiae. From these 80 patients, cultures were taken only sporadically. In paired sera, Eschenbach and coauthors[12] found a fourfold or greater rise in IgG titer in 15 of 74 (20%) AS patients; only five of these 15 were culture-positive for chlamydiae. Paavonen and colleagues[10] found a corresponding titer rise in 19 of 72 (26%), 10 of whom were culture-positive. Thus serologic evidence of an active chlamydial infection in patients with negative cultures is not uncommon among AS patients, even if one takes into consideration the possibility that some patients might have received antibiotic treatment before sampling.

Apart from a group of patients with classical lymphogranuloma venereum and infants with chlamydial pneumonia, patients with AS have the highest levels of antibody found in any group of patients with confirmed *C. trachomatis* infection. Antibodies to chlamydiae also can be detected in cervical secretions in women with chlamydial genital infection.[13] Specific antibody can neutralize the infection of cell monolayers by *C. trachomatis*.[14] Thus a negative cervical culture seems possible in spite of genital chlamydial infection.

In experimental AS in grivet monkeys we found chlamydial infection of the tubes to result in an infiltration, mainly with lymphocytes, in the mucosa (a reaction involving the subepithelial tissues) and partial destruction of the epithelium.[15] We have made a histologic examination of the tubes of two women with chlamydial AS.[16] In both cases the tubal inflammation was intense, with findings similar to those in the experimental infection of the monkeys. When infecting human and bovine oviduct organ cultures with *Chlamydia psittaci* or *C. trachomatis*, Hutchinson and coworkers[17] found no evidence of extensive epithelial cell damage and no loss of ciliary activity, as compared with uninoculated cultures. The reason for this finding could be that only a one-cycle infection was induced and/or that the infection ratio of tubal epithelial cells was low. However, the authors conclude that the changes noted in chlamydial AS represent an immunologic response by the host to chlamydia infection. Corresponding studies on infection of tubal organ cultures with gonococci have shown destruction of tubal mucosa.[18] Hanna and co-workers[19] found that cell-mediated immunity was associated significantly with the presence of antibody in chlamydial infection in man. The findings of a significantly higher GMT of IgG chlamydial antibody in patients with more severe disease might be in accordance with the hypothesis that the tubal destruction in chlamydial AS is a result of immunopathology. In trachoma an active immune response at times has been found to produce more severe disease.[20]

The most severe sequela to AS is postinfection infertility, a result of tubal occlusion.[21] The only prevention available at present is to hinder the infection from reaching the upper genital tract. *C. trachomatis* is one of the major etiologic agents of nongonococcal sexually transmitted disease; in addition, *C. trachomatis* infection is in many countries even more common than gonorrhea. Moreover, the fertility prognosis after nongonococcal AS is worse than that after gonococcal AS.[21] Epidemiologic measures instituted against chlamydial LGTI in men and women should be just as extensive as those against gonorrhea.

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Gonococcal infection in women with pelvic inflammatory disease in Lusaka, Zambia

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Gonococcal infection is the most common disease in both male and female patients seen at the Sexually Transmitted Disease Clinic at the University Teaching Hospital in Lusaka, the capital city of Zambia. In this study, 100 women admitted to the gynecology wards for management of pelvic inflammatory disease were screened for gonococcal infection using an endocervical culture on modified Thayer-Martin medium. Forty-six of these women had gonococcal infection, including 65% of those women with PID of less than 2 weeks' duration. Because gonorrhea is so common among women with PID, "standard" antibiotic regimens must be reevaluated. We recommend for countries with a high incidence of gonorrhea that initial treatment of PID should include an antibiotic regimen effective for gonococcal infection. (AM. J. OBSTET. GYNECOL. 138:965, 1980.)

IN AFRICAN countries where statistics are available[1-3] gonococcal infection has been common and Zambia is no exception. Among men and women attending the Sexually Transmitted Disease (STD) Clinic at the University Teaching Hospital, Lusaka, gonorrhea was the most common STD in men and women, with a prevalence of 27% and 18.8%, respectively.[4] Among symptomatic pregnant women seen in the STD Clinic gonorrhea was identified in 22.9%, and in asymptomatic pregnant women attending University Teaching Hospital antenatal clinics, gonorrhea was identified in 11.2% (Table I, unpublished data).

The serious complication, pelvic inflammatory disease, occurs in 10% to 17% of women who harbor gonococcal infection.[5] Since the incidence of gonorrhea is high in many developing countries of Africa, PID is a common problem in these countries. In addition, inadequate measures to find and treat women in the early stages of infection probably result in an even higher proportion of infected women who develop PID.

The purpose of this study was to assess the rate of gonococcal infection in PID patients admitted to the gynecology wards at University Teaching Hospital and to highlight certain management problems and epidemiologic features of such cases.

Materials and methods

From a large number of patients admitted to the gynecology wards at University Teaching Hospital for treatment of PID during a period of 6 months, 100 were selected for study after excluding women who were pregnant, had delivered or aborted recently, or had undergone gynecologic surgery. In the last category exception was made for three patients who had previously undergone an operation for ectopic pregnancy. Patients also were excluded if they had received antibiotics for the current illness.

All patients were examined in the STD Clinic where the clinical diagnosis of PID was confirmed by findings of abdominal pain and definite pelvic tenderness during bimanual examination. Detailed histories were obtained before a complete genital examination was made. Vaginal discharge was suspended in saline and examined for motile *Trichomonas vaginalis* and elements of *Candida albicans*. After cleaning the ectocervix, material was obtained from the endocervix with a platinum loop. Gram-stained smears were prepared and examined for gonococci. Endocervical material was plated directly onto modified Thayer-Martin medium[6] and incubated immediately in candle jars at 36° C. The cultures were examined after 24 and 48 hours' incubation. Typical oxidase-reactive colonies containing gram-negative diplococci were presumptively iden-

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Table I. Gonorrhea screening in women attending University Teaching Hospital (UTH), Lusaka

Patient group	*No. screened for gonorrhea*	*% With gonorrhea on culture*
STD Clinic	1,000	18.8
Pregnant women referred to STD Clinic for symptoms	170*	22.9
Routine UTH antenatal attendees	161*	11.2

*Unpublished data.

Table II. Results of laboratory tests performed on 100 patients hospitalized for PID at University Teaching Hospital, Lusaka

Laboratory tests	*% Patients with a positive test*
Wet smears of vaginal material for:	
Trichomonads	27.0
Candida	3.0
Gram-stained endocervical smear for gonococci	28.0
Culture for *N. gonorrhoeae*	46.0
Leukocyte count >8,000/mm^3	70.0
ESR >30 mm/hr	74.0
Syphilis serology	
VDRL slide test	13.0
TPHA	12.0

tified as *Neisseria gonorrhoeae*. Blood samples were collected for leukocyte counts, erythrocyte and sedimentation rates (ESRs), and the serologic tests for syphilis—VDRL* slide test and TPHA† test. Leukocytecounts and ESRs were done in the routine hospital laboratory while all other tests were performed in the STD Clinic laboratory.

Results

During the 6 month study period 812 patients with PID were admitted to gynecology wards of University Teaching Hospital (26.5% of all admissions). Postabortion, postpartum, and postsurgical infections accounted for 240 cases. Our study group of 100 patients were part of the remaining 572 cases (18.6% of gynecology ward admissions).

Patient ages ranged from 15 to 43 years with 65% between 20 and 30 years of age. Thirty-eight patients were unmarried; 27 of these were already mothers. Only eight nulliparous women were included in the study group. Multiple sexual partners were noted by 39 patients, including all but two of the unmarried women. Eleven patients were using contraceptive pills, and an intrauterine device (IUD) was in place in eight patients.

Fifty-eight patients had previously had PID, 11 had been secondarily infertile for more than 2 years, and three patients had had ectopic pregnancies.

Trichomonads were seen in 27 patients and yeast was identified in three cases (Table II).

Gonococci were identified in smears from 28 patients and cultures were positive in 46 cases. Leukocyte count was more than 8,000/mm^3 in 70 and ESR exceeded 30 mm in 74. VDRL was reactive in 13 cases, and in 12 the TPHA was also positive.

Many differences were noted between the women with gonococcal PID and those with nongonococcal PID (Table III). Women with gonococcal PID were younger and had more severe disease of more recent onset. Patients with nongonococcal PID were more likely to have had two or more prior episodes of PID. We found no difference in marital status, the number with multiple sexual partners, or relationship of symptom to onset to menstruation.

Discussion

Forty-six percent of these patients with PID had gonococcal infection. This finding is consistent with the high prevalence of gonorrhea among STD Clinic patients and pregnant women in our hospital (Table I). Only 46 women were evaluated within 2 weeks of symptom onset. Among the women with acute PID, 30 of 46 (65%) had gonococcal infection.

Since the sensitivity of a single endocervical culture on a selective medium is only about 80%, the actual incidence of gonorrhea among our patients is even higher than we observed. A previous study from this hospital of 50 PID cases revealed gonococcal infection in only 23.1%.[7] However, this study included women with postabortion and postpartum PID and employed less than optimal culture techniques.

Although others have observed that among women with PID IUDs are used more commonly than any other contraceptive method, we did not find this.[5, 8] However, it is interesting to note that of eight patients with IUDs six had gonococcal infection.

In about one third of women with gonococcal PID, symptoms began during menstruation, and in another third, they started within 5 days following the period. However, the onset symptoms among women with nongonococcal PID was not significantly different.

The number of patients with previous PID was not significantly different in the two groups, although women with gonococcal PID had relatively few previous episodes. Anecdotal observations suggest that re-

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*Venereal Disease Research Laboratories.

†*Treponema pallidum* hemagglutination antibody.

Table III. Comparison of hospitalized women with gonococcal and nongonococcal PID seen at University Teaching Hospital, Lusaka

	Gonococcal PID (n = 46)	*Nongonococcal PID (n = 54)*	*Statistical significance* $p < 0.05$
Age			
15-20 yr	13	8	Yes
21-30 yr	28	37	Yes
>30 yr	5	9	Yes
Unmarried	17 (10*)	21 (17*)	NS†
Multiple sexual partners	18	21	NS
Contraception used			
Pill	6	5	NS
IUD	6	2	Yes
Symptom onset during or within 5 days of menstruation	31 (15‡)	26 (13‡)	NS
Previous PID			
None	28	30	NS
Once	10	8	NS
≥Twice	8	16	Yes
Clinical severity			
Mild to moderate	15	28	Yes
Severe	31	26	Yes
Duration of symptoms			
<2 wk	38	28	Yes
≥2 wk	8	26	Yes
Cervical discharge appearance			
Scanty and mucoid	13	33	Yes
Purulent	33	21	Yes
Leukocyte count >10,000	32	10	Yes
ESR >50 mm	31	16	Yes

*Unmarried mothers.
†Not significant.
‡Onset during menstruation.

curring PID due to gonococcal infection is not uncommon in Lusaka. For example, one patient was treated successfully for acute gonococcal PID but returned with a similar episode within 3 months, when gonococci were again identified.

Gonococcal PID caused more severe symptoms and led to more prompt hospitalization than nongonococcal PID. In addition, patients with gonococcal PID were more likely to have a purulent endocervical discharge and high leukocyte count and ESR. Despite these statistically significant differences, clinical findings of gonococcal PID are less distinctive when compared with the clinical findings of gonococcal urethritis in men.

In a number of studies of PID in Africa[9, 10] and elsewhere,[5] prevalence of gonococcal disease has ranged from 25% to 85%. While sample bias may account for certain differences, gonorrhea appears to be the most common causative agent of PID. Gonococcal infection also predisposes to recurrent PID by other organisms. The frequency of occurrence of gonorrhea and the enormous social and economic consequences of gonococcal PID demand urgent consideration. The need to prevent PID by employing effective diagnostic techniques and promptly administering adequate therapy cannot be overemphasized.

In Zambia, women with PID are not treated routinely with methods that are effective for gonococcal PID. We believe the situation in many other developing countries is no better. Although an appropriate antibiotic may be used, the "standard dosages" often are inadequate to cure gonococcal infections. These regimens may alleviate the symptoms in some cases but will not cure the patient. In addition these inadequate dosages select for antibiotic-resistant gonococcal strains. Hence, in places with a high incidence of gonorrhea, such as Lusaka, we recommend that initial treatment of PID include an antibiotic regimen effective for gonococcal infection.

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Pelvic inflammatory disease and puerperal sepsis in Ethiopia

I. Etiology

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Among 100 outpatient women with symptoms and signs of pelvic inflammatory disease and 200 asymptomatic, postpartum women, the prevalence rates of *Neisseria gonorrhoeae* were 19% and 9%, respectively. Of 46 women hospitalized for PID, 33% were culture-negative, 22% grew *Enterobacteriaceae*, 20% grew streptococci, 15% grew gonococci, and 15% grew anaerobes from blood, pus, or abscesses. One third of 67 women hospitalized for puerperal sepsis (PPS) were culture-negative; the bacteria isolated most frequently from the uterus, blood, urine, and pus were *Enterobacteriaceae* (36%), gonococci (28%), streptococci (25%), and anaerobes (4%). The low frequency of isolation of anaerobes in women with PID and PPS was due largely to limitations placed on the collection and processing of specimens for anaerobic bacteriology. (AM. J. OBSTET. GYNECOL. 138:969, 1980.)

PELVIC INFLAMMATORY DISEASE and puerperal sepsis (PPS) are major causes of both outpatient and inpatient hospital morbidity and death in Ethiopia. A survey of hospital records of 1,329 women admitted to the gynecology wards of St. Paul's Hospital, Addis Ababa, between January, 1973, and May, 1975, revealed that 397 (29.7%) had PID and another 132 (9.9%) had PPS. Twelve of these 529 patients died of their infections, and the remainder were hospitalized for an average of 12.4 days. A similar pattern of illness was seen in the hospital's outpatient clinics. Among 1,153 randomly selected women attending the outpatient gynecology clinic, 450 (39%) complained of lower abdominal or pelvic pain, 415 (36%) of menstrual irregularity, and 150 (13%) of infertility. On pelvic examination, 496 (36%) had thickened fallopian tubes or masses consistent with acute or chronic PID.* Lack of obstetric services and hence delivery at home frequently resulted in women presenting on an emergency basis with a diagnosis of obstructed labor, intrauterine infection, ruptured uterus, or fistula formation, causing gross pelvic sepsis and peritonitis.

The economic and social consequences of PID and PPS are severe. Many of these patients require operation and prolonged hospitalization. Infertility often leads to divorce and maternal death often orphans children. Because of the known association between PID and *Neisseria gonorrhoeae*, we conducted a study to determine the prevalence of this organism in women attending an outpatient gynecology clinic who had symptoms and signs of PID. We also determined the prevalence of asymptomatic gonorrhea in women who were delivered of infants in the hospital. Finally, we

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The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of the Navy.

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*Duncan, M. E.: Unpublished data.

Table I. Isolation of bacteria in patients with PID and PPS

	PID	*PPS*
No. of patients	46	67
Culture positive	31 (67.3%)	45 (67.2%)
One site	20	24
Multiple sites	11	21
Same organism at multiple sites	8	15
Different organisms		
One site	4	4
Multiple sites	3	6

made a more detailed study of the bacterial etiology of disease in hospitalized PID and PPS patients to determine which of the available antibiotics were likely to be the most effective in treating these conditions.

Materials and methods

Patient population. Gonorrhea prevalence studies were conducted on 100 consecutive women with PID attending the outpatient gynecology clinic at St. Paul's Hospital and on 200 consecutive asymptomatic, postpartum women. More detailed bacteriologic cultures were obtained from 46 women hospitalized for severe PID and 67 women requiring continued hospitalization for treatment of PPS. The diagnosis of PID was made in patients complaining of lower abdominal pain, fever, infertility, or abnormal vaginal bleeding who had pelvic or adnexal tenderness and masses suggestive of abscess formation. A diagnosis of PPS was made when a patient had a sustained oral temperature of 38° C or greater for more than 48 hours after delivery and if obvious extragenital causes of infection could be excluded.

Bacteriologic techniques. Specimens for *N. gonorrhoeae* were obtained from the endocervix and anal canal by use of cotton-tipped swabs and from the urethra by use of calcium alginate swabs (Calgiswabs, Inolex, Glenwood, Illinois). In PPS patients, the cervical os was cleaned first, and a sterile cotton swab inserted into the uterus. Swabs were transported in Stuart's medium,[1] inoculated on Thayer-Martin,[2] blood agar, and MacConkey's media, and incubated in 5% CO_2 at 36° C. *N. gonorrhoeae* were identified by colony morphology, oxidase reaction, Gram stain, and the oxidation of glucose, but not maltose or lactose.[3] Aerobic bacteria cultured from the uterus were reported as the organism(s) predominant in the culture.

Blood cultures (Columbia Broth, BBL BioQuest, Cockeysville, Maryland) were obtained before starting antibiotic treatment in patients admitted with PID or PPS. Peritoneal fluid, pus, and pathologic tissue obtained at operation or by culdocentesis were collected in glass syringes or by sterile cotton-tipped applicator sticks and immediately cultured for aerobes on Thayer-Martin and MacConkey's media, and for anaerobes on prereduced anaerobe blood agar (BBL) containing kanamycin (100 μg/ml) and vancomycin (7.5 μg/ml) as well as on thioglycolate medium supplemented with 10% normal rabbit serum. Anaerobic cultures were placed in a jar (GasPak, BBL BioQuest) and incubated at 37° C for a minimum of 7 days. Aerobic and anaerobic isolates were identified by standard techniques using morphologic, culture, and biochemical properties.[3, 4] Smears of pus and peritoneal fluid also were placed on glass microscope slides, Gram stained, and examined for bacteria. Selected aerobic and anaerobic isolates were tested in vitro by the Kirby-Bauer method[5] for their susceptibility to 10 different antibiotics. After admission cultures were collected, most patients were treated with penicillin in combination with either chloramphenicol or streptomycin.[6]

Results

Prevalence of *N. gonorrhoeae*. Of the 100 outpatients with symptoms and signs of PID, 19% had positive genital tract cultures for *N. gonorrhoeae*. The prevalence of *N. gonorrhoeae* was 9% in 200 asymptomatic, postpartum women.

Puerperal sepsis. The 67 women with puerperal sepsis ranged in age from 15 to 28 years (mean, 23.7). In only 45 (67.2%) were one or more bacterial pathogens isolated from either blood, the endocervix, uterus, or abscesses (Tables I and II). The organisms isolated most frequently were *Enterobacteriaceae* (36%), *N. gonorrhoeae* (28%), *Streptococcus species* (25%), and anaerobes (4%).

Ten patients had received antibiotics within 72 hours of delivery, but only two were among the culture-negative group (Table III). The type of antibiotic(s) given to these patients was generally unknown, but *N. gonorrhoeae* was not recovered and group A β-hemolytic streptococci were found significantly less often than in other patients. In three patients different organisms were isolated from different culture sites, and four other patients had polymicrobial endometritis (Table I).

Complicating illness in patients with PPS included nine with peritonitis; one admitted with obstructed labor and ruptured uterus died of *E. coli* septicemia 1 day after laparotomy (Table IV). Another patient had fatal uterine gas gangrene after a late second-trimester abortion. Three patients developed pelvic abscesses after delivery.

Of 19 PPS patients infected with *N. gonorrhoeae*, two were delivered of stillborn infants. Three of the 17

Table II. Bacteria isolated from 45 women with PPS

Bacteria	*No. culture-positive patients*	*Culture site*						
		Uterus	*Pathologic tissue*	*Blood*	*Pus or abscess*	*Urine*	*Other**	*Total*
Enterobacteriaceae								
E. coli	16	6	1	1	4	4		16
Klebsiella species	3	1			1	1		3
Proteus species	3	1			1	1		3
Enterobacter	2	1						2
N. gonorrhoeae	19	16	3				17	36
Streptococci								
S. viridans	5	1	1	2	1	2		7
β-Hemolytic A	7	5	2	1	2	1		11
β-Hemolytic, other	2	1		1				2
S. pneumonia	2	1		1		1		3
Enterococcus	1	1						1
Bacteroides species	3		1		2			3
Moraxella species	2							
Miscellaneous	4			1		3		4
Totals		34	8	9	11	14	17	93

*Rectal and urethral cultures for *N. gonorrhoeae.*

live-born infants had positive gonococcal conjunctival cultures. These three infants and all others born of PPS mothers with gonococcal infection were treated with benzylpenicillin (100,000 U/kg); none developed gonococcal ophthalmia.

PID. Forty-six patients ranging in age from 19 to 48 (mean, 25.8) years were hospitalized for PID. In only 31 (67.3%) were one or more bacterial pathogens isolated from cultures of the endocervix, peritoneal fluid, or blood (Tables I and V). *Enterobacteriaceae* (22%), *Streptococcus species* (20%), *N. gonorrhoeae* (15%), and anaerobes (15%) were the most commonly isolated pathogens. In four patients two organisms were isolated from abscesses: two cases of *E. coli* and *Bacteroides species;* one case of *Proteus species* with *S. aureus;* and one of *Proteus species* and *S. viridans.*

Eleven patients had received some form of antibiotic treatment within 72 hours of admission but only one of these patients was culture-negative at all sites. No gonococci were isolated from patients who had received antibiotics before culture (Table III).

Twenty-nine of the 46 PID patients (63%) had complicating conditions (Table IV). Forty-one (90%) had chronic infections with abscess formation; peritonitis was present in nine (20%). Unusual causes of pelvic abscesses included two patients with ovarian cysts infected by *S. typhi*; neither patient had a clinical history of typhoid fever. Three patients had tuberculous tubo-ovarian abscesses; one of these also had pulmonary tuberculosis. One patient died of a massive pulmonary embolus after laparotomy and another died of first-trimester postabortion uterine gas gangrene.

Table III. Bacteria isolated from 21 patients with pelvic infection given antibiotics before culture

	PID	*PPS*
No. of patients	11	10
No. culture-positive	10	8
Bacteria isolated		
N. gonorrhoeae	0	0
S. viridans	1	2
Streptococci, group A	1	1
Streptococci, other	0	3
E. coli	2	1
S. typhi	1	0
Moraxella species	1	0
S. aureus	2	0
Bacteriodes species	2	1
Clostridia species	1	0

Antimicrobial sensitivity testing. Antimicrobial sensitivity tests were done on all isolates except *N. gonorrhoeae, S. pyogenes,* and *S. pneumonia.* The tested organisms from both PID and PPS patients were most sensitive to chloramphenicol, clindamycin, cephalothin, ampicillin, gentamicin, and tetracycline (Table VI).

Discussion

N. gonorrhoeae has been isolated from about one third to one half of patients with acute PID, with the highest recovery rate during the initial episode and a lower recovery rate with recurrences.[7, 8] *N. gonorrhoeae* probably is the primary cause of genital sepsis in Ethiopia, despite its low recovery rate (15% to 19%) in our PID patients. During their present illness 40% of our outpatients and 24% of our inpatients with PID gave a

Table IV. Complicating conditions in 113 women with pelvic infection admitted for study at St. Paul's Hospital, Addis Ababa, Ethiopia

Primary diagnosis	*No. cases*	*No. complications*	*Description*
Puerperal sepsis or endometritis	67	22	Peritonitis* (9); pelvic abscess (2); pneumonia (3); infectious hepatitis (2); Bartholin abscess (2); single cases of pulmonary and pelvic tuberculosis, infected episiotomy, and gas gangrene*
Acute salpingitis	5	2	Primary syphilis (2)
Pelvic abscess	41	27	Peritonitis (9); pelvic (3) and/or pulmonary tuberculosis; ovarian cyst (6); abortion* (4); uterine fibroids (2); genital ulcers (2); pulmonary embolus* (1)
Total	113	53	

*One case fatality.

Table V. Bacteria isolated from 31 women with PID

		Culture site					
Bacteria	*No. culture-positive patients*	*Endocervix*	*Pus or abscess*	*Blood*	*Pathologic tissue*	*Other**	*Total*
N. gonorrhoeae	7	6	1			5	12
Enterobacteriaceae							
E. coli	5		4		1	7	12
Proteus species	2		2			5	7
Enterobacter species	1				1		1
S. typhi	2		1		1		2
Streptococci							
S. viridans	5		4	1	1		6
β-Hemolytic, non–group A	3		2			1	3
S. pneumonia	1		1				1
Moraxella species	1		1	1			2
S. aureus	2		1		1		2
Anaerobes							
Bacteriodes species	4		4				4
Clostridia species	2		1	1			2
Peptostreptococcus	1		1				2
M. tuberculosis	3		3				3
Miscellaneous	4		3	2			5
Totals		6	29	5	5	18	63

*Urine and urethral or rectal culture for *N. gonorrhoeae.*

history of self-medication or prior antibiotic treatment. Prior antibiotic therapy influences the type of bacteria isolated, especially *N. gonorrhoeae*. By eliminating those patients who received antibiotics, the frequency of isolation of *N. gonorrhoeae* would increase to 20% to 32% in our PID patients. Eliminating patients who received antibiotics would likewise increase the recovery rate of gonococci from 28% to 33% in PPS patients. Our gonococcal recovery rate was greater than the 4% observed by Brown and Cruickshank[9] among a comparable group of women with PID in Rhodesia, but lower than the 43% recovery rate at Kenyatta National Hospital in Nairobi, Kenya.[10]

Of great concern was the frequent isolation of *N. gonorrhoeae* both from asymptomatic parturient women and from those with puerperal sepsis. The former went untreated, since they were discharged the day after delivery, before the results of culture were known. These patients were almost impossible to locate and thus received treatment for gonorrhea only if they developed puerperal sepsis or if their infants developed ophthalmia neonatorum, prompting hospital attendance.

The etiologic significance of bacteria isolated from sites other than blood or abscesses was uncertain, particularly in PPS patients. Culture specimens taken from the uteri of PPS patients contained varying numbers of different aerobic bacteria, and the bacteriologist made an arbitrary decision about which, if any, of the bacteria isolated were to be reported. Moreover, anaerobic cultures were not done routinely in PPS patients, although anaerobes are the predominant bacteria present in the uterus in the immediate postpartum period.[11] The majority of our PPS patients had a polymicrobial endometritis, which was initiated by retained

Table VI. Antimicrobial sensitivity of bacteria causing PIP or PPS at St. Paul's Hospital

Drug	Total isolates tested	% Isolates		
		Sensitive	Intermediate	Resistant
Penicillin	43	55.8	18.6	25.6
Streptomycin	58	29.3	12.0	58.7
Chloramphenicol	59	81.4	1.6	17.0
Clindamycin	32	81.3	3.1	15.6
Tetracycline	60	68.3	3.3	28.4
Cephalothin	59	88.1	3.4	8.5
Ampicillin	55	78.2	5.5	16.3
Kanamycin	59	52.5	5.1	42.4
Gentamicin	58	70.7	3.4	25.9
Sulfa	41	56.1	—	43.9

placental tissue or blood clots. Most of these women were delivered of their infants at home, attended only by traditional midwives, and could not be given oxytocic drugs to prevent postpartum uterine hemorrhage or retention of blood clots and placental tissue.

The frequency of isolation of anaerobic bacteria from abscesses in PID and PPS patients was low and does not accurately reflect the presence of these organisms in peritoneal fluid or pus. For example, about half the abscess material was fetid at operation and often contained gram-negative rods by Gram stain, but was culture-negative for both anaerobic and aerobic bacteria. This phenomenon probably was due to improper collecting and processing of anaerobic specimens or to the bactericidal effects of antibiotics administered before specimen collections. We were reluctant to perform surgery on any patient without prior antibiotic treatment because of the risks and sequelae of bacteremia during and after an operation.

Further definition of the etiology of PID will require laparoscopic techniques for collecting specimens and sophisticated bacteriologic techniques for processing them. Laparoscopy for Ethiopian women with PID, however, may be complicated by a high incidence of intraabdominal adhesions from prior peritonitis. In Addis Ababa, this procedure carries the additional possible risk of carbon dioxide narcosis since the geographic elevation is 8,200 feet.

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Genital tuberculosis in women

Analysis of 187 newly diagnosed cases from 47 Swedish hospitals during the ten-year period 1968 to 1977

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A retrospective analysis of symptomatology, diagnostic procedures, and treatment of all 155 new cases of genital tuberculosis at 47 Swedish gynecology departments in the period 1968-77 was performed. The frequency of genital tuberculosis was 0.002% of all patients admitted for gynecologic disease. Genital tuberculosis occurred more frequently in the postmenopausal period. The most common symptoms were metorrhagia, pain, and infertility. Chemotherapy alone was used in 40% of the cases. Conservative surgery was attempted in 8%, and 38% had radical surgery. No intrauterine and four tubal pregnancies occurred after therapy. We conclude that primary treatment should be conservative, although the chances of having a normal pregnancy are almost nil and the risk of an ectopic pregnancy is great. (AM. J. OBSTET. GYNECOL. 138:974, 1980.)

A FEW DECADES AGO, genital tuberculosis in women was very common and was considered as a possible diagnosis in every gynecologic investigation.[1, 2] During the past decade, however, the disease has become increasingly rare and is now so uncommon that present-day gynecologists, at least in Sweden, hardly expect to find it. The literature has been sparse also and limited to reports on small series of cases.[3-5] In a review published in 1976, Schaefer[6] pointed out the importance of bearing the disease in mind at diagnosis, particularly since it occurs mainly in younger age groups. In this study, 80% to 90% of the patients were women of childbearing age. He also stated that involuntary childlessness and abdominal pain were the most common reasons for patients seeking consultation. Abnormal vaginal bleeding, on the other hand, was relatively uncommon (about 20% of the cases). Among infertile women, Schaefer[6] found that between 5% and 10% of the cases of infertility were due to tuberculosis. For a long time, the general impression in Sweden was that the clinical picture had changed. We considered it of interest to analyze a large series of cases in order to evaluate the clinical situation.

Methods

Since an individual hospital will admit only a few patients with genital tuberculosis, we conducted a retrospective survey of all 57 departments of gynecology in Sweden. We asked them to provide us with complete photocopies of the case records for all patients with newly detected genital tuberculosis during the 10 year period, 1968 through 1977. Thus we obtained the best possible information on patient symptoms, diagnostic procedures, treatment, follow-up, and reasons for consultation.

As a minimum criterion for diagnosis of genital tuberculosis, we required the presence of typical tuberculous granuloma in pathologic specimens. We did not require a positive bacteriologic diagnosis since most clinics did not require such before starting treatment.

Results

We received replies from 47 (82%) of the gynecology services. Altogether, 187 patients with a diagnosis of genital tuberculosis were treated in the 10 year period. Among the responding hospitals, 28 reported that a total of 573,216 women were hospitalized with gynecologic diseases. The incidence of genital tuberculosis in these patients was two cases per 10,000 gynecologic admissions (i.e., 113 cases).

The age distribution is shown in Fig. 1. If the patients are divided into two 5 year periods, 1968-72 and

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Table I. Number of patients older and younger than 50 years of age at the onset of genital tuberculosis, 1968-72 and 1973-77*

	Age				
	<50 yr		*≥50 yr*		
Yr	*N*	*%*	*N*	*%*	*Total*
1968-72	63	55	51	46	114
1973-77	28	38	45	62	73
Total	91	49	96	51	187

*$p < 0.05$.

Table II. Site of the infection in 72 patients with previously diagnosed tuberculosis (TB) from a group of 187 women with genital TB, 1968-77

Site of infection	*No. of patients*
Lung	54
Kidney	3
Cervical lymph glands	3
Peritoneum	5
Intestine	1
Eye	2
Bone	4
Total	72 (38%)

Table III. Presenting complaints or other reasons for consultation in 187 women with genital tuberculosis

Symptoms	*No. of patients*	*%*
No symptoms (health control)	21	11.2
Pain including dysmenorrhea	46	24.6
Bleeding	77	41.2
Infertility	24	12.8
Other diseases	19	10.2
Total	187	100.0

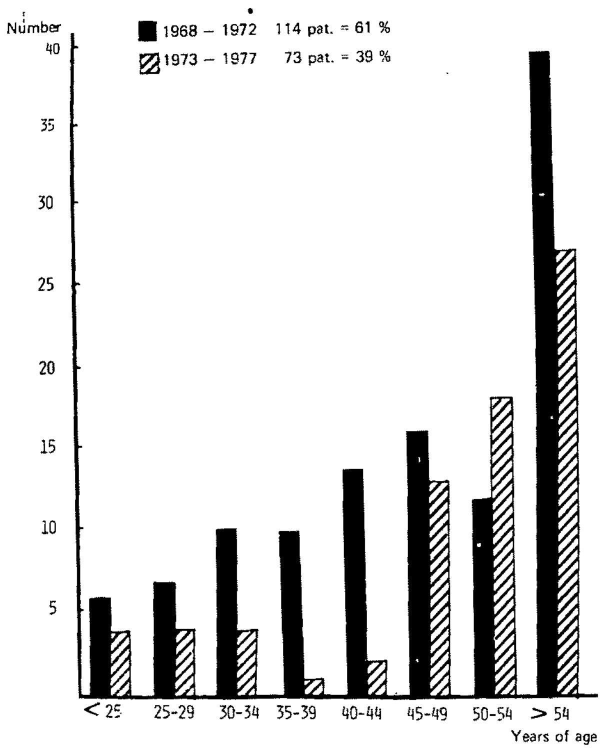

Fig. 1. Age distribution of 187 women with diagnosed genital tuberculosis during the periods 1968-72 and 1973-77.

1973-77, the number of cases decreases from 114 during the first period to 73 during the second. The disease occurs more commonly after childbearing age; there is a shift toward greater age during the second 5 year period. Forty-six percent of the patients were 50 years of age or older during the first period, as compared to 62% during the second period. The difference is statistically significant at the 5% level (Table I).

Genital tuberculosis is considered to be secondary to tuberculosis in other organs, usually the lungs. Thirty-eight percent of the patients had previously diagnosed tuberculosis (Table II).

Symptoms or other factors leading to the diagnosis are shown in Table III. The most common symptom was abnormal bleeding. Many patients (11.2%) were completely asymptomatic or were found to have the disease in connection with investigations for other disorders. Table IV lists the other diagnoses found among the cases in our study. Among these, there were eight patients with carcinoma, three of whom had the uncommon diagnosis of carcinoma tubi.) There were two patients with tubal pregnancies. In most of the patients, bimanual examination was normal. In 46 patients, however, palpable masses could be detected in the pelvis minor. As already stated, five cases of cancer of the adnexa and two benign ovarian tumors were included in these patients. The remainder had inflammatory masses. Hysterosalpingography was done in 36 patients, but tuberculous salpingitis was suspected in only six of these cases.

In most patients the diagnosis was based on pathologic investigation of the endometrium (Table V). A bacteriologic diagnosis was obtained in only 55 cases (29%). In 50 cases a pathologic diagnosis was not obtained until after extirpation or resection of the fallopian tubes. In most of these cases, tuberculosis had not been suspected and was found unexpectedly at microscopic examination of extirpated material.

The predominant treatment for all patients was chemotherapy. In addition, 101 patients were subjected to surgery. In many cases (65.3%), the diagnosis was not established preoperatively and the patient un-

Table IV. Other coincident diseases in 187 patients with genital tuberculosis

Disease	*No. of patients*
Fibromyoma	3
Cystadenoma of ovary	2
Carcinoma of ovary	2
Carcinoma of tube	3
Carcinoma of endometrium	3
Tubal pregnancy	2

Table V. Diagnostic findings in 187* women with genital tuberculosis (TB)

Findings	*No. of patients*
Endometrial biopsy	
With TB granulomas	130
And with positive culture	44
TB granulomas in tubes	50
TB granulomas in cervix	1
Positive culture from menstrual blood, abscess, etc.	11

*Total in table does not equal 187 owing to multiple findings in some of the patients.

Table VI. Preoperative diagnosis in 101 patients with known or later proven genital tuberculosis (TB)

Diagnosis	*No. of patients*
Known TB, drug-treated	35
Tumor	24
Adnexal mass	16
Infertility	8
Tubal pregnancy	2
Peritonitis	2
Other diagnosis	14
Total	101

Table VII. Treatment of 187 women with genital tuberculosis*

Treatment	*No. of patients*	*%*
No treatment	4	2.1
Drug treatment only	67	35.8
Drug treatment and radical surgery	53	24.6
Drug treatment and bilateral salpingectomy	26	14.5
Drug treatment and conservative surgery	22	10.7
Incomplete information	15	12.3
Total	187	100.0

*Drug treatment was started before operation if diagnosis was known. In other cases drug treatment was started as soon as diagnosis of tuberculosis was established.

Table VIII. End result after treatment of 139 women with genital tuberculosis*

		Drug treatment combined with	
	Drug treatment only	*Hysterectomy and bilateral salpingectomy*	*Conservative surgery*
Symptomless	40	39	26
Pain and/or bleeding	12	3	5
Reop. because of sequelae	—	—	4
Op. ileus	—	—	1
Tubal pregnancy	1	—	—
3 Tubal pregnancies	1	—	—
Dead from intercurrent disease	—	3	—

*Result in 48 women unknown.

derwent operation for reasons other than known tuberculosis (Table VI). When tuberculosis was diagnosed before operation, chemotherapy was administered preoperatively. When the diagnosis was established only after the operation, chemotherapy was instituted as soon as possible.

Some patients (10.7%) were subjected to conservative surgery aimed at preserving tubal function (Table VII). For 15 patients the records are so vague that it is not possible to determine with certainty what type of treatment the patients received.

In this retrospective analysis of case records from departments of gynecology, it proved difficult in certain instances to determine the results of the treatment. Responsibility for treatment often was taken over by a chest physician, and the patients were not always followed up by a gynecologist. However, Table VIII lists the results in 139 women whose case records contained sufficient information. One hundred and five (76%) of these patients became free of symptoms. There were no intrauterine pregnancies, but we found four tubal pregnancies, three of them in the same patient.

Discussion

The picture has changed completely since 1950, when Jedberg[1] concluded that genital tuberculosis was diagnosed during childbearing age in 98% of all women with this disease. Most cases are now diagnosed after menopause. Consequently, patients with problems of involuntary childlessness no longer predominate. Instead, the most common symptoms are bleeding and pain. It still is important to remember, however, that tuberculosis may be the cause of infertility. Consideration of history of tuberculosis must not be neglected. It is evidently very difficult for present-day radiologists and gynecologists to recognize the often typical radiologic picture of tuberculosis at hysterosalpingography. It is therefore important to include curettage and a chest x-ray when investigating patients for infertility even when tuberculosis is not suspected at hysterosalpingography. Our analysis of case records showed that this often was neglected in the preoperative investigation. As a rule, however, reconstructive surgery is meaningless in patients with tubercular salpingitis. Furthermore, there is a risk of a latent infection and subsequent relapse.[7] If the pathologist suspects tuberculosis, repeated tuberculosis cultures from the endometrium and menstrual blood should be performed, preferably before treatment is started. It would seem reasonable, however, to start treatment before the results of the cultures are available since other causes of the typical granuloma usually seen at the pathoanatomic investigation usually can be excluded.[4]

The primary treatment should be antibiotics. This mode of treatment is so effective nowadays that surgery should be carried out only on the following indications: (1) persistent masses in the pelvis minor after antibiotic treatment—note that such masses may be neoplasms, (2) relapse after treatment, and (3) persistent pain after treatment. If surgery is performed, it should be radical, preferably with both hysterectomy and bilateral salpingectomy. In premenopausal women, however, the ovaries should be preserved unless they are involved in the infection.

Jedberg[1] pointed out that pregnancy should be regarded as a complication. This still applies. The only pregnancies in this study were tubal pregnancies. Therefore, the wish to preserve fertility should not be allowed to interfere with surgery. Although a few pregnancies in patients with minor lesions caused by genital tuberculosis have been reported, these patients, in principle, should be regarded as infertile.[6] A patient need not suffer three tubal pregnancies before undergoing radical surgery.

Finally, these patients naturally should be followed up with regular controls by a gynecologist. Consultation with chest physicians regarding antibiotic treatment should, of course, be utilized, but it is the gynecologist's duty to follow the results of the treatment and judge the need for surgery. The gynecologist also helps the patient to cope with fertility and sexual problems in connection with the disease.

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Gonococcal salpingitis is less likely to recur with *Neisseria gonorrhoeae* of the same prinicipal outer membrane protein antigenic type

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If protective immunity were to develop following an episode of gonococcal pelvic inflammatory disease (salpingitis), PID should recur with organisms bearing antigens that do not react with these immune mechanisms. To test this hypothesis, gonococci from 15 women with gonococcal PID, who experienced 19 subsequent episodes of gonococcal infection, were serotyped for their principal outer membrane protein (Protein I) antigens. Of nine cases in which the initial and subsequent infections involved the same Protein I serotype, none was associated with recurrence of PID. Of 10 cases in which the initial and subsequent infections involved different Protein I types, five were associated with recurrent PID (p = 0.02). These data suggest that an episode of gonococcal PID produces some immunity to repeated episodes of salpingitis with the same Protein I serotype, while not preventing reinfection with the same Protein I serotype. The immune response to Protein I antigen may thus provide serotype-specific protection against gonococcal salpingitis. (AM. J. OBSTET. GYNECOL. 138:978, 1980.)

PATIENTS WITH salpingitis or pelvic inflammatory disease caused by *Neisseria gonorrhoeae* frequently have high antibody responses to gonococcal antigens, presumably owing to potent immunizing stimuli from their invasive infection.[1-3] These antibodies may reflect a potent host immune response that might protect against subsequent salpingitis caused by antigenically similar gonococci. Prominent surface antigens of *N. gonorrhoeae*, that also interact with bactericidal antibodies, are lipopolysaccharide (LPS) and principal outer membrane protein (Protein I).[4-7] Any protective immunity that developed following salpingitis might be expected to interact with these gonococcal surface antigens. If protective immunity were dependent upon bactericidal antibodies, one might observe protection against recurrent salpingitis with organisms of the same LPS or Protein I serotype, without concomitant protection against recurrent localized mucosal infections. This is because bactericidal antibodies do not prevent mucosal infections,[3, 8] presumably as a result of inadequate complement levels in genital secretions.[9] To test the correlation between Protein I serotype and recurrent infection with or without salpingitis, we determined the Protein I serotype of all gonococci isolated from 15 patients who experienced an initial episode of gonococcal PID, followed by 19 episodes of recurrent gonococcal infection either with (five patients) or without (14 patients) salpingitis.

Materials and methods

Study population. Approximately 300 patients with PID were examined by one of us (D. E.) at Harborview Medical Center or the University Hospital in Seattle, and followed at frequent intervals after treatment for 1 to 3 years. Approximately half of this group initially were found to have gonococcal PID and about 30 of these patients experienced recurrent gonococcal infec-

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This research was supported in part by United States Public Health Service contracts NO1-AI-52535 and NO1-AI-92618, by grants RO1-AI-13149 and AI-12192 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, by a grant from the World Health Organization, and by Federal Health Program Service Project SEA 76-43.

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tion in the follow-up period. Fifteen of these patients met the criteria for inclusion in this study. These criteria were (1) an initial episode of salpingitis caused by *N. gonorrhoeae*, followed by one or more subsequent gonococcal infections 3 weeks to 18 months (median, 2 months) after the initial infection, (2) sufficient clinical and laboratory data available for each clinic visit to determine whether the patient had salpingitis, a localized mucosal infection, or no infection, and (3) availability of all isolates of *N. gonorrhoeae* from each patient for Protein I serotyping. Patients were considered to have recurrent infections with or without salpingitis only if it could be demonstrated that their cultures were negative for *N. gonorrhoeae*, and that they were without signs or symptoms of PID, for a period after treatment of their initial gonococcal PID and prior to its recurrence. Cases were classified as uncomplicated reinfections if the patient had mild or no adnexal or cervical motion tenderness at the time of reinfection. All patients with recurrent gonococcal PID had moderate to severe adnexal or cervical motion tenderness in association with recurrent endocervical gonococcal infection.

Protein I serotyping. The Protein I serotype of each gonococcal isolate was determined by a quantitative enzyme-linked immunosorbent assay (ELISA) in which purified Protein I was coated to polystyrene tubes. The optical density developed through reacting rabbit antibody to Protein I, followed by goat antirabbit antibody linked to enzyme, followed by enzyme substrate and color reagent, was significantly inhibited only by gonococci possessing Protein I of the same serotype on their surfaces.[7, 10] The specifics of our ELISA methodology have been published previously.[11] A total of nine Protein I serotypes (1 through 9) have been identified, and each of the 15 initial and 19 subsequent isolates of *N. gonorrhoeae* was tested in duplicate against each of the nine Protein I serotypes (612 tests). Also, 10 additional isolates of *N. gonorrhoeae* from patients experiencing their first episode of salpingitis were tested in duplicate against the same nine Protein I serotypes (180 tests). This provided 25 total isolates from which to characterize the prevalence of different Protein I serotypes on gonococci causing PID in the nonimmune patient in Seattle. If an isolate typed with more than one Protein I serotype, it was considered the same serotype as any other isolates with which it shared ≥50% of its Protein I serotypes.

Results and discussion

- None of the nine reinfections with gonococci of the same Protein I type as the organisms that caused the initial PID was associated wth PID. Of 10 reinfections with gonococci of Protein I serotypes different than the initial PID strain serotype, five were associated with recurrent PID (Table I, $p = 0.022$). This is consistent with the hypothesis that an immune response directed at least in part at Protein I antigens develops during gonococcal PID, and that this immunity protects against recurrent salpingitis, but not necessarily against localized mucosal infections caused by gonococci of the same Protein I serotype. These data provide a rationale for further evaluation of Protein I antigens as candidates for inclusion in a vaccine to protect against gonococcal salpingitis. This evaluation would include tests of the safety and immunogenicity of Protein I antigens in man, as well as determination of the frequency of different Protein I serotypes on gonococci causing PID.

Table I. Protein I serotype of gonococci that reinfected patients after a previous episode of gonococcal PID

Protein I serotype	*Reinfection*	
	With PID	*Without PID*
Same*	0	9
Different	5	5

*As compared to the Protein I serotype of the gonococci that caused the initial episode of PID.

Table II. Protein I serotypes* of *N. gonorrhoeae* causing salpingitis in 25 women with first-episode PID

Serotype	%
1	68%
2	60%
3	0%
4	4%
5	16%
6	20%
7	16%
8	32%
9	0%

*Protein I serotypes 1 and 2 are strongly related and all but four isolates that typed with either of these Protein I antigens also typed with the other related Protein I serotype. In addition, some other isolates typed with more than a single Protein I serotype.

The prevalence of Protein I serotypes on gonococci causing salpingitis in 25 nonimmune patients from Seattle is summarized in Table II. Most of the isolates were of serotypes 1 (64%) and 4 (64%), followed by Protein I types 8 (32%), 6 (20%), and 5 and 7 (16%). A Protein I vaccine containing serotypes 1 and 2 would have encompassed 72% of the strains, and one containing serotypes 1, 2, and 8 would have covered 84% of the 25 strains causing PID in our study. Strains from other geographic areas are needed to better assess the extent of heterogeneity of Protein I serotypes of gonococci causing salpingitis. These preliminary results offer some basis for hope that a vaccine based upon Protein I antigens can be developed to protect against gonococcal salpingitis.

Sylvia Cowles, Ronald Kroft, Linda Sires, Kathy Duffin, Mike Weaver, and Tom Bukowski provided valuable technical assistance.

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Gonococcal lipopolysaccharide: A toxin for human fallopian tube mucosa

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Gonococci damaged the mucosa of human fallopian tubes in organ culture (FTOC), producing characteristic pathologic features. Filter-sterilized supernatant fluid from donor gonococcal-infected FTOC damaged recipient FTOC in a similar fashion. Gonococcal lipopolysaccharide (LPS) was detected in these toxic donor fluids in concentrations of 1.2 to 8.3 μg/ml. Purified gonococcal LPS in concentrations as low as 0.015 μg/ml produced damage equivalent to that caused by toxic donor fluid and was neutralized by polymyxin B. Such LPS-mediated damage to ciliated cells, if it occurs in gonococcal salpingitis, may impair mucociliary flow and predispose to ectopic pregnancy and recurrent ascending infection. (AM. J. OBSTET. GYNECOL. 138:981, 1980.)

GONOCOCCAL INFECTIONS remain epidemic in many parts of the world. The disease may spread from local genital sites to the fallopian tubes, perihepatic area, joints, and heart valves with crippling or life-threatening consequences. Even after eradication of gonococcal infection of the fallopian tubes, there is a residual predilection to pelvic inflammatory disease, ectopic pregnancy, and sterility.[1] In addition, approximately 80% of the annual economic costs of gonococcal infection are related directly to gonococcal infection of the fallopian tubes. Despite this enormous problem, the mechanisms by which gonococci damage human tissues in general and fallopian tubes in particular have been investigated only recently.

Study of gonococcal virulence factors has been difficult because of the relative specificity of gonococcal infections for man. To circumvent this difficulty we have employed an experimental model of gonococcal infection of human fallopian tubes in organ culture.[2] This model allows quantitation of the effect of virulence factors and correlation of dynamic and pathologic changes that occur in gonococcal infection. Previous studies in our laboratory demonstrated that gonococci damaged the mucosa of human fallopian tubes in organ culture, producing characteristic pathologic features.[3] Filter-sterilized supernatant fluid from donor gonococcal-infected organ cultures rapidly damaged recipient fresh organ cultures in a similar fashion. This and other observations suggested that at least part of the damage to human genital mucosa by gonococci was mediated by a subcellular toxic factor or factors. In the studies reported here, use of the human fallopian tube organ culture model has extended our previous investigations[3-5] of the characteristics of the toxic factor(s) and has provided evidence that gonococcal lipopolysaccharide (LPS) is the principal factor mediating this toxic damage.

Materials and methods

Microorganisms. *Neisseria gonorrhoeae* strain 2686, colony type 1, was used in these studies. Microorganisms were subcultured selectively as described previously,[3] and were consistently of transparent colony type.[6]

Preparation of human fallopian tube organ cultures (FTOC). Fallopian tubes were obtained from perimenopausal, nonpregnant women and maintained in HEPES minimum essential medium (HEPES MEM) as described previously.[3]

Infection of organ cultures and preparation of donor organ culture supernatant fluid. Infection of FTOC, preparation of donor organ culture superna-

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These studies were supported by United States Public Health Service Research Grant AI-13488 from the National Institute of Allergy and Infectious Diseases. Dr. Gregg participated in these studies while a recipient of National Research Service Award AI-05533 from the National Institute of Allergy and Infectious Diseases.

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Table I. Dose-related toxic effect of gonococcal LPS on human fallopian tube mucosa

LPS concentration (μg/ml)	*PPCA**
15	0.53 (0.04)†
1.5	0.56 (0.04)
0.15	0.48 (0.06)
0.015	0.55 (0.08)
0.0015	0.82 (0.05)
0.00015	0.91 (0.03)
0.000015	0.93

*Each value represents the mean ratio of the PPCA (percentage of the periphery with ciliary activity) remaining at 24 hours, relative to the control value at the same interval.

†Figures in parentheses represent the standard error of the mean.

tant fluid, and preparation of recipient FTOC for assessment of toxic damage were performed as described previously.[2, 3] For experiments in which gonococcal LPS was tested for its effects on fallopian tube mucosa, lyophilized LPS was dissolved in pyrogen-free water with sonication. This solution was diluted appropriately in HEPES MEM and then filtered through a 0.45 μm membrane filter (Millipore Corp.) prior to testing in the FTOC model.

Quantitation of mucosal damage. Damage to the fallopian tube mucosa was quantitated as the percentage of the periphery with ciliary activity remaining at each period of observation, as described previously.[2]

Effect of dialysis on toxicity of donor fluid. Supernatant fluid from fallopian tube organ cultures infected with gonococci for 48 hours was filter sterilized. An aliquot was dialyzed against 1 L of fresh HEPES MEM for 20 hours at 4° C. This dialyzed fluid was then tested in recipient FTOC and compared to undialyzed toxic supernatant donor fluid.

Effect of heat on toxicity of donor fluid. Supernatant fluid from 48 hours infected donor FTOC was filter sterilized and was left unheated or was heated at 56° C or 85° C for 30 minutes. These fluids were then tested for their ability to damage recipient FTOC.

Absorption of toxic supernatant fluid with *Limulus* amebocyte lysate (LAL). Pooled samples of filter-sterilized supernatant fluid from uninfected FTOC and from gonococcal-infected FTOC were absorbed with LAL as described elsewhere.[7] LAL assays were performed on all samples before and after absorption. Prior to use as the medium for recipient FTOC, these fluids were sonicated for 15 minutes and diluted 1:4 with fresh HEPES MEM, as in the standard recipient experiments.

Preparation of purified gonococcal LPS. Purified gonococcal LPS was prepared by a modification of the hot phenol-water technique described by Johnson and Perry.[8]

Mouse lethality studies. Gonococcal LPS was tested for its lethal effects by intravenous injection into lead-sensitized mice, essentially as described by Rifkind.[9]

Quantitation of endotoxic activity. The endotoxic activity of FTOC supernatants was determined by the LAL assay. Purified gonococcal LPS served as the standard for determination of endotoxic activity. *Limulus* lysate, *Escherichia coli* LPS control standard, and methodology were obtained from Associates of Cape Cod, Inc., Woods Hole, Massachusetts.

Polymyxin B studies. Polymyxin B (Burroughs Wellcome & Co.) was tested for its ability to neutralize the toxic effects of gonococcal LPS on FTOC. Polymyxin B, 3 μg/ml, was incubated with gonococcal LPS, 0.15 μg/ml, for 30 minutes at 25° C. Then this mixture was used as the medium for FTOC in parallel with appropriate controls.

Results

Effect of dialysis and heat on toxic activity. Most of the toxic activity of donor supernatant fluid remained after dialysis. Neither heating at 56° C nor at 85° C for 30 minutes diminished the toxic activity of donor fluid.

Studies of the source of the toxic factor(s). The supernatant fluid collected from uninfected FTOC homogenized in its own medium was not toxic for recipient FTOC. Gonococci grown in broth culture (in the absence of fallopian tube tissue) elaborated substantial quantities of material that was toxic for FTOC. These observations, plus the finding that the toxic factor was heat-stable, suggested that the toxic activity detected in gonococcal-infected donor FTOC fluids was mediated by a product of the gonococcus and not of the host tissue.

Effect of absorption with LAL on toxic activity. Toxic supernatant fluid absorbed with LAL had significantly less damaging effect than unabsorbed toxic donor fluid when compared in recipient FTOC. LAL assay of these toxic fluids before and after absorption disclosed that LAL removed >99% of the measurable endotoxic activity.

Preparation of purified gonococcal LPS. The lot of LPS used in these studies was free of nucleic acid contamination and contained less than 1% protein. The lot of LPS used in the mouse lethality studies contained less than 1% protein and approximately 4% ribonucleic acid.

Mouse lethality studies. Mice that received LPS but no lead acetate were not killed by gonococcal LPS at a dose of 20 μg. However, in those mice that were lead-sensitized prior to receiving gonococcal LPS, the median lethal dose of gonococcal LPS was 0.07 μg.

Quantitation of LPS. Purified gonococcal LPS in concentrations of 0.006 ng/ml consistently caused gelation of LAL. This endpoint was used as a standard for measurement of gonococcal LPS concentrations in FTOC supernatants. Whereas LPS was not detected in control FTOC fluids, toxic supernatants from 48 hour gonococcal-infected FTOC contained LPS in concentrations ranging from 1.2 to 8.3 μg/ml.

Dose-related toxic effect of gonococcal LPS. In preliminary experiments, purified gonococcal LPS was tested at a concentration of 50 μg/ml in FTOC. At this LPS concentration, the damage to the fallopian tube ciliary activity occurred at a rate equivalent to that seen in recipients of toxic FTOC donor fluids.

Tenfold dilutions of gonococcal LPS ranging from 15 to 0.000015 μg/ml were prepared in HEPES MEM and tested for their toxic effects in FTOC. LPS concentrations as low as 0.015 μg/ml consistently caused maximal damage. LPS at a concentration of 0.0015 μg/ml caused measurable but submaximal damage, but LPS of a concentration ≤0.00015 μg/ml did not cause significant damage (Table I).

Effect of polymyxin B in blocking the toxic effect of gonococcal LPS. In three experiments, polymyxin B was preincubated with gonococcal LPS. In one of these experiments polymyxin B totally neutralized the toxic effect of LPS on FTOC. In the other two experiments there was significant blocking of the toxic effect.

Discussion

A major problem in studying the virulence of gonococci has been the virtual specificity of the genital infection for man and, thus, the lack of a convenient experimental model of genital infection with gonococci. To help overcome this problem Ward and associates[10], Carney and Taylor-Robinson,[11] and McGee and colleagues[2] have developed experimental models of human fallopian tubes in organ culture for studying the interaction of gonococci with human genital tissue. Previous studies using this model demonstrated that gonococcal damage to human fallopian tube mucosa was correlated with loss of ciliated cells and subsequent invasion of nonciliated cells.[3] Furthermore, it was shown that colony type 4 gonococci damaged tissues, although at a lower rate than type 1 organisms, indicating that factors other than pili are involved in virulence. Two observations suggested that gonococci elaborate a toxic factor (or factors) which is at least partially responsible for the damage to the mucosa: (1) Considerable damage occurred prior to entry of gonococci into mucosal cells, and (2) the primary damage was due to sloughing of ciliated cells although gonococci attached almost exclusively to nonciliated cells. Additional studies demonstrated that filter-sterilized supernatant fluid from donor gonococcal-infected fallopian tube organ cultures damaged fresh recipient fallopian tube organ cultures.[3] The studies reported here expand our previous observations and provide evidence that gonococcal LPS is the principal mediator of this toxic activity.

The toxic activity of donor fluids from gonococcal-infected FTOC was not reduced by heating to 85° C and was reduced only partially by dialysis. These data suggest that the principal toxic factor in these fluids is heat-stable and has a molecular weight of greater than 12,000 daltons. These physical properties are characteristic of gonococcal LPS. Mårdh and his group[12] found that gonococcal LPS impaired the mucociliary wave frequency of human fallopian tube organ cultures. In our studies toxic activity in the donor fluids was diminished as LPS was removed by absorption with LAL. These experiments suggested that gonococcal LPS plays a key role in mediating the observed toxic damage to human fallopian tube mucosa.

Purified gonococcal LPS was prepared and tested for its endotoxic effects. This LPS was highly lethal in lead-sensitized mice. Furthermore, purified gonococcal LPS consistently caused gelation of LAL when mixed at a concentration of 0.006 ng/ml with the lysate. Purified gonococcal LPS at concentrations comparable to those of toxic donor fluid damaged FTOC at a rate equivalent to that caused by toxic donor fluids. In further studies, the toxic effect of gonococcal LPS was produced by LPS concentrations as low as 0.015 μg/ml. These studies confirmed that the gonococcal LPS concentrations in toxic donor organ culture fluids greatly exceeded those necessary to account for the damage these fluids cause in recipient FTOC.

A probe for investigating the toxic moiety of LPS has been provided by the demonstration that the antibiotic polymyxin B neutralizes a variety of biologic effects of lipopolysaccharides in vitro[13] and in experimental animals,[9, 14] probably by binding with and blocking the lipid A portion of the molecule.[15] Our studies demonstrated that polymyxin B significantly blocked the toxic effects of gonococcal LPS on FTOC. These studies suggest that the toxic moiety of gonococcal LPS, as with endotoxins of other gram-negative bacteria, is the lipid A portion of the molecule. Further studies are in progress to elucidate the structure of this gonococcal LPS and to investigate the biologic properties of its component parts.

Gonococci elaborate into their milieu outer membrane material with endotoxic properties.[16] Our studies demonstrate that gonococcal LPS is capable of causing damage to ciliated cells of human fallopian tube mucosa. Such LPS-mediated damage to ciliated cells, if it occurs in gonococcal salpingitis, may compromise mucociliary flow and predispose to ectopic pregnancy and recurrent ascending infection.

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Microbiology and pathogenesis of acute salpingitis as determined by laparoscopy: What is the appropriate site to sample?

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Acute salpingitis is a polymicrobial disease. *Neisseria gonorrhoeae* and anaerobic gram-positive cocci were the predominant microorganisms isolated from the fallopian tubes of salpingitis patients. Gonococci were isolated from the fallopian tubes in eight of 35 (23%) patients; anaerobic bacteria were recovered from 10 of 35 (28.5%). Although *Chlamydia trachomatis* was not recovered from the fallopian tube exudate, there was abundant serologic evidence of chlamydial infection in the salpingitis patients. Twenty-three percent of patients with paired sera had a fourfold rise in IgM and IgG titer, which was consistent with systemic chlamydial infection. Comparison of cultures obtained via laparoscopy and culdocentesis suggested that culdocentesis is not an accurate reflection of the microbial milieu in the fallopian tube. (AM. J. OBSTET. GYNECOL. 138:985, 1980.)

ACUTE SALPINGITIS is the major medical and economic consequence of uncomplicated anogenital gonorrhea in women.[1] Other microorganisms also have been implicated as causative agents in this disease.[2-9] Acute salpingitis is a direct cause of infertility, leads to increased reproductive wastage secondary to ectopic pregnancies, and may result in other sequelae and inflammary residua necessitating surgical intervention.[10]

Based on the recovery of *Neisseria gonorrhoeae* from the endocervix, the gonococcus has been implicated as the etiologic agent of acute salpingitis in 33% to 80% of patients.[1, 2-7] However, investigations utilizing culdocentesis and/or laparoscopy to obtain cultures from the peritoneal cavity and tubal exudate have shown a variable correlation between the cervical and intraabdominal cultures.[2-7] In these studies the gonococcus could be recovered from the peritoneal cavity in only 6% to 80% of salpingitis patients with endocervical gonorrhea. Previous investigations have reported the isolation of a variety of anaerobic and aerobic bacteria from the peritoneal fluid.[2-7] Thus, controversy has arisen over the role of the gonococcus in the etiology and pathogenesis of acute salpingitis. In addition, microorganisms such as *Chlamydia trachomatis* and genital tract mycoplasmas have been recovered from the peritoneal cavities of salpingitis patients.[6-9]

Prevention of significant morbidity and sequelae and reduction of costs associated with acute salpingitis require development of appropriate methods of treatment and prevention that are based on knowledge of the microbiologic etiology and pathogenesis of the disease. This study was undertaken to determine the microbiologic etiology at the site of infection—the fallopian tube. In a previous study[6] we noted a discrepancy between culdocentesis isolates and fallopian tube isolates. The recovery of microorganisms in the culdocentesis specimen that were not isolated directly from the fallopian tube exudate cast doubt on the assumption that bacteriologic evaluation of peritoneal fluid speci-

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Supported in part by research grants AI 15642, AI 16631, HD 13462, and HD 12271 from the National Institutes of Health.

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Table I. Distribution of microorganisms isolated in the SFGH* Acute Salpingitis Study

Microorganism	*Fallopian tube (N = 35)*	*Culdocentesis (N = 35)*	*Endocervix (N = 39)*
N. gonorrhoeae	8 (23%)	11 (31%)	19 (49%)
N. gonorrhoeae only	6 (17%)	3 (8.5%)	—
N. gonorrhoeae in combination with other bacteria	2 (6%)	8 (23%)	—
Aerobic bacteria only	1 (3%)	1 (3%)	—
Anaerobic bacteria	10 (29%)	20 (57%)	—
Anaerobes only	3 (8.5%)	5 (14%)	—
Mixed anaerobes/aerobes	7 (20%)	15 (43%)	—
M. hominis	0	1 (3%)	28 (80%)
Ureaplasma urealyticum	3 (8.5%)	6 (17%)	21 (60%)
C. trachomatis	0	0	2 (5%)
No growth	13 (37%)	9 (26%)	—

*San Francisco General Hospital.

Table II. Microorganisms isolated from the fallopian tube and culdocentesis aspirate in SFGH Acute Salpingitis Study, N = 35

Microorganisms	*Fallopian tube*	*Culdocentesis aspirate*
Aerobic organisms		
Haemophilus influenzae	2	1
Haemophilus species	1	0
Streptococcus	3	6
Group B streptococcus	0	2
Cornybacterium vaginale	1	7
Enterococcus	0	1
Escherichia coli	1	3
Proteus mirabilis	0	1
Staph. epidermidis	1	1
Pseudomonas aeruginosa	1	1
Diphtheroids	1	0
Lactobacillus	0	3
Anaerobic organisms		
Peptococcus	4	3
Peptostreptococcus	3	6
Bacteroides oralis	0	1
Bacteroides bivius	0	1
Bacteroides melaninogenicus	0	1
Gram-negative rod	0	1
Fusobacterium	1	3
Veillonella	0	1
Clostridium	1	3
Gaffkya	0	2
Ruminococcus	0	2
Propionibacterium	0	2
Diphtheroids	0	1

Table III. Microbial isolates from the fallopian tubes, cul-de-sac, and culdocentesis aspirate of acute salpingitis patients (N = 10)

Organism	*Cul-de-sac by laparoscopy*	*Fallopian tube by laparoscopy*	*Culdocentesis*
Aerobes	9	8	10
Anaerobes	4	6	12
Gonococcus	2	2	3
Ureaplasma urealyticum	2	1	2
Fungi	0	0	1
Chlamydia	0	0	0
Total	17	17	28

mens obtained via culdocentesis is an accurate reflection of the microorganisms present in the fallopian tube of salpingitis patients. Thus caution should be used in interpreting bacteriologic specimens obtained via culdocentesis. Therefore, our second goal was to determine whether culdocentesis accurately reflects the microbiologic milieu of the fallopian tube or if its usefulness is limited by contamination with vaginal microorganisms. Third, we set out to assess the role of *C. trachomatis* in acute salpingitis.

Materials and methods

Thirty-nine women were enrolled in the study. These patients presented with a tentative diagnosis of salpingitis, gave informed written consent, and had subsequent laparoscopic confirmation of acute salpingitis.

The criteria for the diagnosis of acute salpingitis have been described previously.[6] In the initial 29 patients, cultures were obtained from three anatomic sites—endocervix, cul-de-sac via culdocentesis, and fallopian tube exudate via laparoscopy. In 10 additional patients, a fourth culture site also was utilized—the cul-de-sac via laparoscopy. The fallopian tube exudate, laparoscopically obtained cul-de-sac sample, and culdocentesis specimen were cultured for *N. gonorrhoeae, Mycoplasma hominis, Ureaplasma urealyticum, Mycoplasma fermentans,* aerobic and anaerobic bacteria, herpesvirus, and *Chlamydia trachomatis.* The endocervix was cultured only for *N. gonorrhoeae,* genital mycoplasma, *Ureaplasma,* herpesvirus, and *C. trachomatis.* The microbiologic methodology has been described previously.[6] Statistical analysis was performed using Fisher's exact test.

Table IV. Microbial isolates from patients with acute salpingitis

Patient	*Cul-de-sac by laparoscopy*	*Fallopian tube by laparoscopy*	*Culdocentesis*
1. M. R.	*Corynebacterium vaginale*, aerobic diphtheroids	0	*Corynebacterium vaginale, N. gonorrhoeae, Lactobacillus, Gaffkya anaerobius, Ruminococcus*
2. B. H.	*Ureaplasma urealyticum*	*Ureaplasma urealyticum*	*Ureaplasma urealyticum, C. vaginale,* anaerobic gram-neg. rod, streptococcus, anaerobic diphtheroids
3. V. M.	0	0	Peptostreptococcus, *Bacteroides bivius*
4. C. V.	*Staph. epidermidis*	*Staph. epidermidis,* diphtheroids	*Aureobasidius pullulans*
5. D. G.	Streptococcus, *Pseudomonas aeruginosa, E. coli,* peptostreptococcus, *Bacteroides* species	Streptococcus, *Pseudomonas aeruginosa, E. coli,* peptostreptococcus, *Bacteroides* species, *Clostridium*	Streptococcus, pseudomonas, *E. coli,* peptostreptococcus, *Bacteroides* species
6. D. N.	Aerotic diphtheoirds, peptococcus, peptostreptococcus, streptococcus	*C. vaginale*, aerobic diphtheroids, peptococcus, peptostreptococcus, streptococcus, *Fusobacterium*	*C. vaginale,* peptococcus, peptostreptococcus, *Fusobacterium, Veillonela,* streptococcus, *Ureaplasma urealyticum*
7. A. C.	*Ureaplasma urealyticum, C. vaginale*	0	*Ureaplasma urealyticum*
8. G. B.	0	0	*Staph. epidermidis*
9. A. W.	*N. gonorrhoeae*	*N. gonorrhoeae*	*N. gonorrhoeae*
10. D. G.	*N. gonorrhoeae*	*N. gonorrhoeae*	*N. gonorrhoeae*

Results

Microbiologic etiology. The general distribution of microorganisms isolated from the various culture sites is listed in Table I. Although nearly 50% of the patients had gonococci in the endocervix, the gonococcus could be recovered from the fallopian tube in only eight of 35 (23%) and from the culdocentesis aspirate in 11 of 35 (31%) patients. Of the patients with endocervical gonococci, gonococci were isolated from the fallopian tube in 42% and from the culdocentesis aspirate in 58%. The specific microorganisms obtained from the fallopian tube and by culdocentesis are listed in Table II.

Anaerobic bacteria were the organisms recovered most frequently from the fallopian tube exudate, and they were isolated in 10 cases (28.5%). In the seven patients with mixed aerobic-anaerobic bacteria isolated from the tubal exudate, anaerobes were the predominant organisms. *Peptostreptococcus* and *Peptococcus* species were the most prevalent anaerobes in the tubal exudate. Anaerobic bacteria were recovered from the culdocentesis aspirate in 53% of the patients. In a single instance a sole aerobic organism recovered from the tubal exudate was an enterococcus. The Gram-stained smears showed rare, tiny, pleomorphic gram-positive cocci resembling anaerobic cocci in five of the 13 patients with no growth.

While *M. hominis* and *Ureaplasma urealyticum* were recovered frequently from the endocervices of women with acute salpingitis (Table I), these microorganisms also were recovered frequently from the fallopian tube. Herpesvirus was not recovered from any site.

***Chlamydia trachomatis* in salpingitis patients.** *C. trachomatis* was recovered from the endocervices of two patients (5%). *C. trachomatis* was not isolated from the fallopian tube exudate or cul-de-sac fluid in any patients. However, there was abundant serologic evidence of chlamydial infection in this population. Some of the women showed serial conversion with antibodies detected at high titers, which is consistent with systemic chlamydia infection. Thirty-two of 37 patients tested had some antibodies to *C. trachomatis*. Of 22 women with paired sera, five had a fourfold rise in titers to chlamydia antigens (IgM and IgG). Seven additional patients had very high single-point titers, with IgM ($\geq 1:32$) compatible with acute chlamydial infection. Of the 12 patients with a fourfold rise in titer or single high titers, all had complement fixation titers greater than 1:16. Only one of the remaining 25 patients had a complement fixation titer greater than 1:16 ($p < 0.001$).

Comparison of culdocentesis and fallopian tube isolates. The microorganisms identified in the 10 women in whom cultured specimens were obtained from the fallopian tube, cul-de-sac via laparoscopy, and cul-de-sac via culdocentesis are shown in Table III. Although there was close agreement between fallopian tube exudate and cul-de-sac isolates via laparoscopy, there was poor correlation with the culdocentesis results. Fifty percent more isolates were obtained from the culdocentesis aspirate than from the fallopian tube exudate or cul-de-sac fluid obtained via laparoscopy. A patient-by-patient comparison of isolates is presented in Table IV. The major contributors to the increase were anaerobic bacteria, *Corynebacterium vaginale*, aerobic streptococci, and *E. coli*.

Previously we[11] reported that the colony phenotypes of the fallopian tube/cul-de-sac isolates of gonococci were more transparent than the paired endocervical isolates, which were more opaque. In Patients 9 and 10 (Table IV) the colony phenotypes of the culdocentesis isolates were similar to the phenotypes of the same patients' endocervical isolates.

Discussion

Recent investigations to determine the microbiologic etiology of acute salpingitis have utilized culdocentesis and/or laparoscopy in an attempt to provide culture specimens from the site of infection that are not contaminated by the abundant microflora of the female lower genital tract. These studies have suggested a polymicrobial nature for the etiology of acute salpingitis[2-7] and have questioned the role that *N. gonorrhoeae* plays in the etiology and pathogenesis of the disease. On the one hand, groups led by Chow,[3] Monif,[4] and Cunningham[5] have proposed that symptomatic infection of the upper genital tract is initiated almost always by the gonococcus, which then paves the way for secondary infection with microorganisms from the flora of the cervix and the vagina. In contradistinction, other groups led by Eschenbach,[2] Sweet,[6] Mårdh,[9] and McCormick[12] have suggested that *N. gonorrhoeae* does not always initiate salpingitis, and that nongonococcal salpingitis is a separate clinical entity. Optimal resolution of this controversy would require daily laparoscopically obtained tubal cultures in salpingitis patients not receiving therapy. Obviously this is not possible. In our initial study[6] and in this report, the use of laparoscopy to determine the microorganisms isolated from the fallopian tube exudate has confirmed the polymicrobial etiology of acute salpingitis. In addition, the use of laparoscopy to collect specimens from the fallopian tube exudate has suggested alterations in the concepts of the etiology and pathogenesis of acute salpingitis.[11, 13, 14]

In an attempt to answer this dilemma, we have determined the recovery of *N. gonorrhoeae* and other microorganisms as a function of the duration of salpingitis and the number of salpingitis episodes.[13] Although the gonococcus tended to be recovered from patients early in the clinical course of salpingitis and was the pathogen isolated most frequently from the fallopian tube within the initial 24 hours of symptoms, other bacteria also were present early in the disease process. In three of the patients presenting within 24 hours of onset of symptoms, anaerobic bacteria were the predominant fallopian tube isolates. *N. gonorrhoeae* was not isolated from the fallopian tubes of these patients and was not even recovered from the cervix in one case. Thus it appears that other bacteria from the cervix and vagina may not require *N. gonorrhoeae* to pave their access to the fallopian tube.

We were able to isolate anaerobic bacteria from the fallopian tubes in the initial episode of salpingitis. It was the initial episode in six of 11 patients with anaerobic isolates. In five of these women, anaerobes were the predominant organism, and gonococci were not recovered from the fallopian tube or endocervix. Both gonococci and anaerobes/aerobes were recovered in inverse proportion to the number of episodes of salpingitis.[13] These data suggest that the gonococcus does not necessarily initiate all primary episodes of salpingitis.

The use of laparoscopy has enabled us to study several potential virulence factors in paired fallopian tube/peritoneal cavity and endocervical isolates of *N. gonorrhoeae*.[11, 14] These included auxotypes, antimicrobial susceptibility, and colony phenotype. While the auxotypes of gonococci isolated from salpingitis patients differed from the auxotypes of gonococci from uncomplicated anogenital disease, the auxotypes of paired tubal-endocervical isolates were similar. Despite a relative increased resistance to a variety of antibiotics in the salpingitis gonococci as compared to uncomplicated anogenital gonococci, the paired tubal-endocervical isolates demonstrated similar patterns. The only potential virulence factor that was significantly different in the fallopian tube and endocervical paired gonococcus isolates was colony phenotype. The fallopian tube phenotypes were more transparent than the corresponding endocervical isolates.

Similar to reports by Eschenbach and associates[2] and Mårdh and Weström,[8] tubal isolation of *M. hominis* and *U. urealyticum* was infrequent although we frequently isolated these organisms from the cervices of salpingitis patients. All three tubal isolates of *Ureaplasma* were from patients presenting with symptoms lasting longer than 72 hours. This raises the question as to whether these organisms are secondary invaders rather than primary pathogens.

The role of *C. trachomatis* as a pathogen in acute salpingitis has received attention recently. Although we were unable to isolate this organism from any fallopian tube exudate, there was serologic evidence of systemic chlamydial infection in 23% of our salpingitis patients with paired sera. Mårdh and colleagues[9] reported 30% isolation of *C. trachomatis* from the fallopian tubes. However, in the United States, *C. trachomatis* has been recovered from the peritoneal cavity in only 2% to 10% of salpingitis patients.[2, 7] Serologic evidence suggests that approximately 20% of all salpingitis cases in the United States are associated with this agent. The difference between Swedish isolation rates and those in the United States may be due to the methods of collecting specimens, whereby the Swedes use a needle biopsy aspiration technique, and successful isolation may require the collection of infected cells in the inoculum. Second, the high rate of gonococcal infection in the United States may submerge the role of *Chlamydia trachomatis* in the pathogenesis of salpingitis in this country. Although complement fixation (CF) titers are not a good indicator of cervical or urethral infection with *C. trachomatis*, the correlation of CF titers of 1:16 with IgM titers suggestive of recent systemic *C. trachomatis* infection indicates that CF is a relatively good test in systemic infection.

We noted a lack of correlation between culdocentesis isolates and those obtained from the fallopian tube. Culdocentesis specimens yielded greater numbers of bacteria common to vaginal flora than did the fallopian tube isolates. This suggests vaginal contamination occurs with culdocentesis. Eschenbach and associates[2] suggested that the isolation of vaginal contaminants occurred when cul-de-sac aspirates were cultured in broth, but not on direct plating or Gram-stained smears. However, in this study there was only a single instance of a *Staphylococcus epidermidis*, which grew only on subculture of broth and not on direct plating. There were two instances of growth on the plate but not on broth subculture. The similarity noted between the colony phenotype patterns of the gonococci isolated from the culdocentesis aspirate and the endocervix reinforces the possibility of the culdocentesis results reflecting vaginal contamination. These findings suggest that laparoscopically obtained specimens from the fallopian tube may be the only appropriate material for elucidating the microbiologic etiology of acute salpingitis. In addition, the study of *N. gonorrhoeae* obtained from endocervical canal specimens may not be comparable to the study of fallopian tube isolates. Thus, if we are to understand the pathogenesis of acute salpingitis and determine the virulence factors of the pathogens involved in the disease process, isolates must be studied that have been obtained from the site of infection, i.e., the fallopian tube. Only by elucidating these factors can appropriate methods of treatment and prevention be developed that will avoid the significant morbidity and sequelae, and reduce costs associated with acute salpingitis.

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Experimental pelvic inflammatory disease provoked by *Chlamydia trachomatis* and *Mycoplasma hominis* in grivet monkeys

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Pelvic inflammatory disease was produced by inoculation with *Mycoplasma hominis* and *Chlamydia trachomatis* directly into the fallopian tubes of grivet monkeys. The effects of various routes of infection were examined. The method of inoculation with the infecting agent influenced the resulting clinical spectrum. In these monkeys significant changes in humoral antibodies occurred with inflammation caused by both *M. hominis* and *C. trachomatis*. Development of cellular antibodies against *M. hominis* could be demonstrated in all experimentally infected monkeys, whereas no such antibodies could be detected in monkeys with chlamydia salpingitis. *M. hominis* in female grivet monkeys may spread from the cervix or the uterine cavity, probably via blood vessels and lymphatics, producing parametritis and "exosalpingitis." *C. trachomatis* may spread from the cervix via the uterine mucosa, producing endosalpingitis with destruction of the epithelium. The infection caused by *M. hominis* in the genital tract of female grivet monkeys resembles "nongonococcal" salpingitis while *C. trachomatis* produces infection resembling "gonococcal" salpingitis. (AM. J. OBSTET. GYNECOL. 138:990, 1980.)

OFTEN IN THE PAST, *Neisseria gonorrhoeae* has been considered the prime cause of nontuberculous salpingitis. However, during the last two decades, evidence for an etiologic role of two other sexually transmitted organisms, viz., *Mycoplasma hominis*[4, 7, 15] and *Chlamydia trachomatis*,[3, 12] has been presented.

The grivet monkey was used as an animal model to examine the capacity of *M. hominis* and *C. trachomatis* to produce inflammation of the female upper genital tract. This article reports the results of our experiment.

Material and methods

Animals. The 25 female grivet monkeys *(Cercopithecus aethiops)* used, body weights = 1.6 to 2.4 kg, had been captured in East Africa and kept in quarantine for 6 weeks before experimentation.

Organisms. *M. hominis*, strain D 1887, isolated from the uterine cervix of a patient with acute salpingitis, was used. Before inoculation the organism was handled as described previously.[8]

A strain (No. 771) of *C. trachomatis*, immunotype K, isolated from the fallopian tubes of a patient with acute salpingitis was used. An inoculum of this strain, which was injected into a monkey, produced acute salpingitis. The organism was reisolated from the fallopian tubes of the infected monkey, relabeled (strain No. 5155), and used in the experiments. These isolates were handled as reported elsewhere.[10]

Preinoculation testings. Specimens were collected from all monkeys on three consecutive occasions from the throat, the urethra, the cervix, and the rectum, and were cultured for mycoplasmas, particularly for the occurrence of *Mycoplasma hominis*, *M. fermentans*, and *Ureaplasma urealyticum*.[8] Swab specimens from the same sites also were collected for *C. trachomatis*.[10]

The preinoculation testing also included blood examination and, in some cases, determination of the rectal temperature.

Experimental infection. Different methods were used to inoculate animals with the organisms. The uterine tubes were directly infected during laparotomy, as described earlier.[7] Moreover, studies of the mode of

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This work was supported by grant Nos. 512-10196 and 512-15503 from The Danish Research Council.

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spread of infection from the lower to the upper genital tract were performed following three other modes of inoculation: route A, inoculation into the uterine cavity through a steel catheter inserted into the cervical canal; route B, into the uterine cavity via the cervical canal, but after ligation of the isthmus of the uterine tubes, followed by curettage of the endometrium; or route C, into the epithelium of the cervical mucosa. These procedures have been described in detail elsewhere.[7]

All surgery and sampling were performed after inducing general anesthesia.[8]

Assessment of lesions and sampling were done, with few exceptions, on days 3, 7, 14, 21, and 35 post inoculation (p.i.), and in some cases, on day 60 p.i. On these days laparotomy was performed. Biopsies and/or swab specimens were collected from the fallopian tubes, fimbriae, parametria, and the intestinal serosa for histology and culture studies. On the same occasion, and at varying intervals during the third month p.i., swabs were taken from the cervix.

Culture studies. The methods used for isolation of mycoplasmas, *Ureaplasma*, chlamydiae, and bacteria were the same as those described earlier.[8, 10]

Histology. The tissue biopsies were fixed in 10% formaldehyde, embedded in paraffin, and stained with hematoxylin and eosin.

Serology. Antibody surveys for mycoplasmas were carried out by an indirect hemagglutination assay (IHA).[2] In some of the experiments, serum antibodies also were determined by a modification of the growth inhibition (GI) test, by a modified immunofluorescence (IMF) test, and by a metabolic inhibition (MI) test.[5]

Surveys for serum antibodies to *C. trachomatis* were performed by use of a microimmunofluorescence test, using pools of antigen.[14]

Blast transformation tests. Lymphocytes were obtained from monkey blood separated on Ficoll-Isopaque gradients. Then the cells were washed, counted, and cultured in RPMI 1640 (Gibco) supplemented with 20 mmole HEPES, 100 IU penicillin/ml, 100 μg streptomycin/ml, and 15% heat-inactivated pooled normal human serum. The cultures were made in microtiter plates. Each well contained 0.2 ml of lymphocytes in a concentration of 10^6 cells/ml. Undiluted antigen at 30 or 10 μl and 10 μl of antigen diluted 1:4, 1:16, and 1:64 were added to the cultures. All determinations were done in triplicate. Unstimulated and antigen-stimulated cultures were incubated in 5% CO_2 at 37° C for 4 days. Quantitation was done by adding 0.02 Ci ^{14}C-thymidine to each well 18 hours before termination of the experiments.

Controls. In monkeys used as controls in the *M. hominis* studies, phosphate-buffered saline (PBS), pH 7.2, was the inoculum. A yolk sac suspension from a noninfected embryonated hen's egg, which had been diluted in PBS, was inoculated into the control monkeys used in the *C. trachomatis* experiments.

Results

Gross lesions. The nine monkeys inoculated with *M. hominis* directly into the fallopian tubes, into the uterine cavity after ligature of the isthmus of the tubes (route B), or into the epithelium of the cervical mucosa (route C) all showed obvious signs of inflammation in the upper genital tract on days 7 to 21 p.i. The parametria were swollen, and the tubes were moderately congested and reddened. On day 35 p.i., the internal genital organs were practically normal on macroscopic examination. The monkeys inoculated via the uterine cavity (route A) with *M. hominis* presented normal parametria during the experimental period, whereas the fimbrial ends of the uterine tubes were moderately reddened and swollen on day 7 p.i. Later, the internal organs appeared normal.

When *C. trachomatis* was injected directly into the tubes, into the cavum uteri (route A), or into the cervical epithelium (route C), all seven monkeys developed salpingitis. The entire tubal length was swollen and reddened on days 7 to 21 p.i. Exudate appeared in the abdominal ostia of the tubes and a watery exudate was found in the cul-de-sac. A yellowish discharge was seen in the posterior vaginal fornix from day 7 p.i. and during the following 2 to 3 weeks. Invariably the parametria were normal. The monkeys inoculated with *C. trachomatis* into the uterine cavity after ligature of the tubal isthmuses (route B) presented no inflammatory signs in the genital tract. One of the monkeys in this experiment died on day 14 p.i. Autopsy showed diffuse peritonitis and perihepatitis with adhesions between the peritoneum and the anterior surface of the liver.

Histologic findings. All nine monkeys that showed macroscopic signs of inflammation after infection with *M. hominis*, viz., monkeys injected with inoculum directly into the uterine tubes or by routes B and C, had inflammatory changes in the sections of the parametria and the tubes. The lesions were characterized by edema and hyperemia together with intense infiltration of lymphocytes and a variable number of heterophilic granulocytes in the parametria and in the subserosa of the tubes, extending into the superficial layer of the tubae muscularis. The tubal epithelium was intact, and no exudate or adhesions of the mucosal folds were observed. In the monkeys in which the gross appearance of the internal organs was practically normal, viz.,

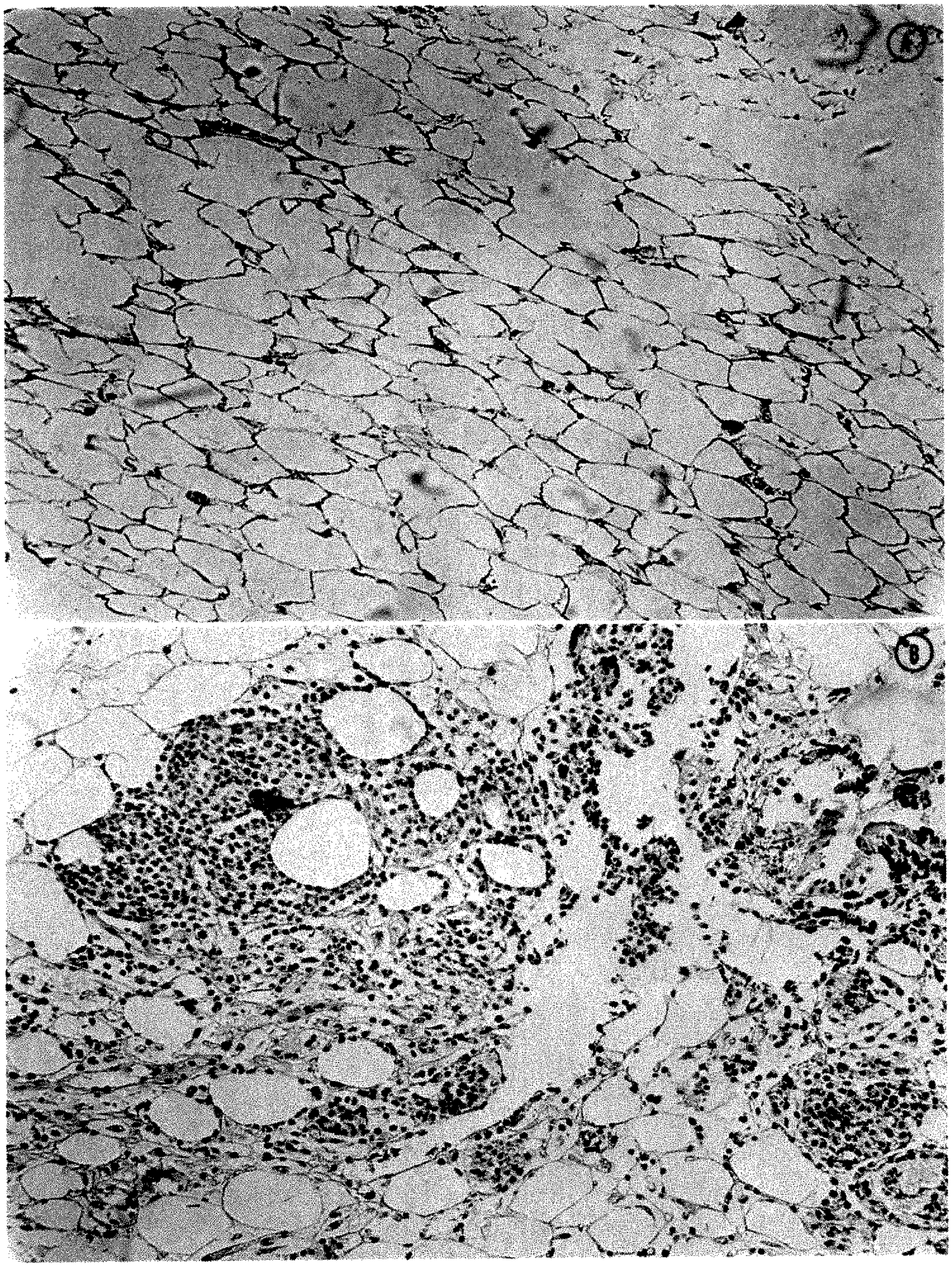

Fig. 1. Section of a parametrium of a monkey infected in the uterine cavity with *M. hominis* after ligature of the tubal isthmuses and curettage of the endometrium *(A)* on the day of inoculation, showing normal parametrium, and *(B)* on day 7 p.i., showing marked inflammatory changes characterized by edema and infiltration with a great number of lymphocytes. (Hematoxylin and eosin. Original magnification × 100.)

monkeys inoculated by route A, there was slight infiltration of leukocytes in the subserosa of the tubes. The alterations of the parametria were characterized by minimal infiltration of lymphocytes in the subserosa (Fig. 1).

In the monkeys in which inoculation of *C. trachomatis* provoked gross lesions of the fallopian tubes, viz., monkeys injected with inoculum directly into the tubes or by routes A or C, the histologic findings were characterized by a marked inflammatory infiltrate involving the mucosal epithelium, which was injured in some areas. An exudate with inflammatory cells and some desquamated cells was present in the lumina of the tubes (Fig. 2).

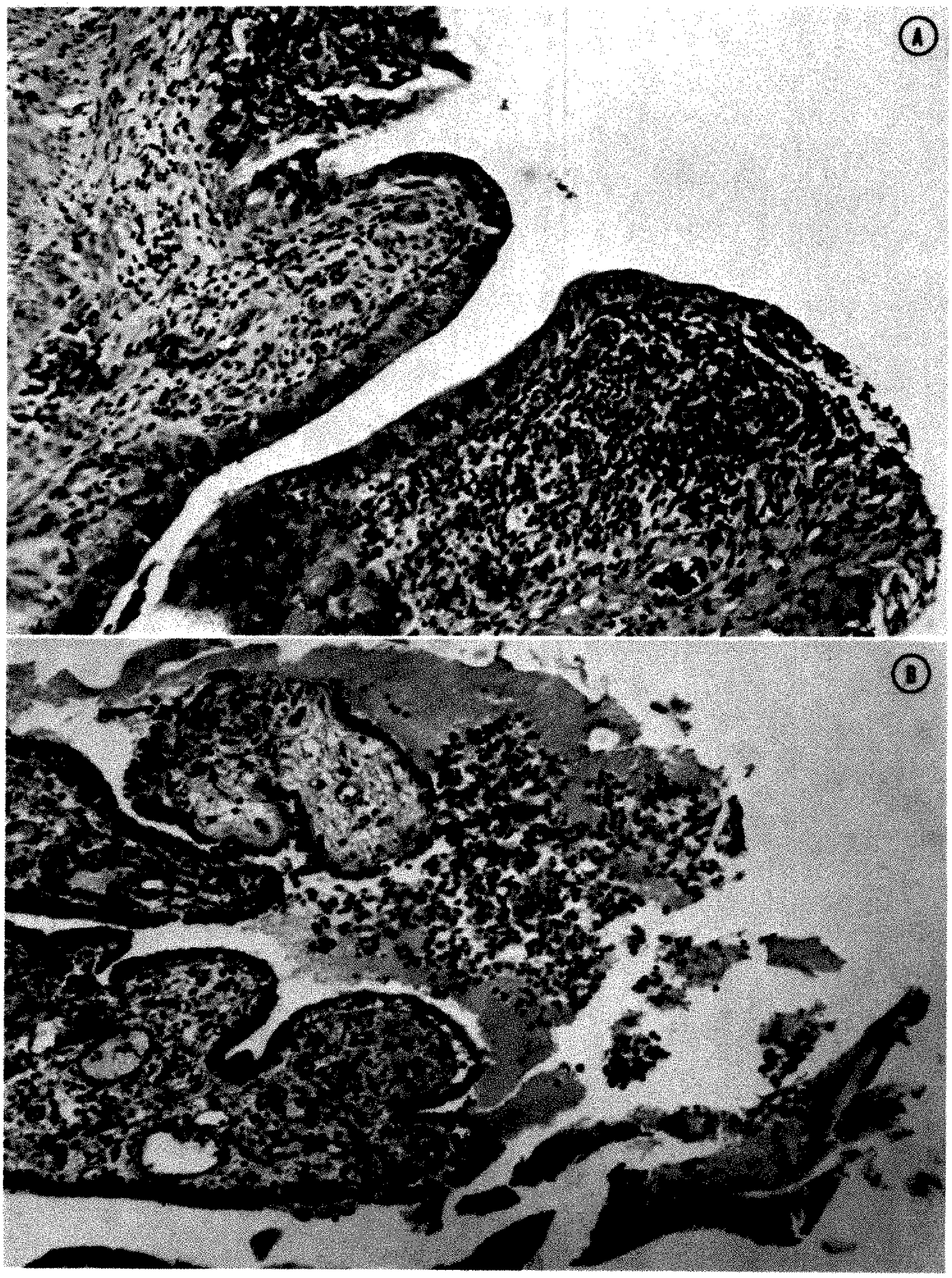

Fig. 2. Section of a tube of a monkey inoculated with *C. trachomatis* into the uterine cavity *(A)* on day 3 p.i., showing pronounced inflammatory infiltrate in the subepithelial tissue and partly in the epithelium, and *(B)* on day 7 p.i., showing pronounced inflammatory infiltrate in the tubal epithelium and in the subepithelial tissue. The epithelium is injured in some areas, and the tubal lumen contains an exudate mostly of lymphocytes and desquamated cells. (Hematoxylin and eosin. Original magnification × 100.)

Two months after the inoculation the lumina of the tubes were diminished, the tubal epithelium was flattened, and adhesions occurred between the mucosal folds in some animals. The parametria showed no microscopic signs of inflammation throughout the investigation period.

Antibody studies. In all monkeys with upper genital tract infection caused by *M. hominis*, IHA antibodies to this organism developed on days 7 to 12 p.i. A fourfold or greater rise in antibody titers occurred during the following 2 weeks. The antibodies persisted for at least 2 months after the inoculation. Different tests for the detection of serum antibodies to *M. hominis* in the grivet monkeys were compared. These tests included IHA, MI, IMF, and modified CI tests. The IHA was found to be superior to the other methods used.[5] Thus, a four-fold rise in IHA antibody titers was observed in all monkeys, the increase first being demonstrated on days 7 to 12 p.i. Maximum titers of 160 to 640 occurred on day 20 p.i. This antibody level persisted to day 60 p.i. The modified GI and the IMF tests also showed a rise in the titers, although less pronounced than that demonstrated by the IHA. A faster decrease in the titer of GI and IMF antibodies was found also. It was not possible to detect *M. hominis* antibodies in the sera of any of the monkeys by use of the MI test.

Antibodies against *C. trachomatis* developed in all monkeys in which salpingitis occurred. Characteristically, a primary antibody response occurred with an IgM to IgG conversion. The IgG antibodies persisted 2 months after the inoculation while the IgM antibodies disappeared after 4 to 6 weeks.

Cell-mediated immunity. A rise in cell-mediated antibody, as measured by the blast transformation test, was found as early as day 7 after the inoculation in all monkeys that developed mycoplasma parametritis. It persisted for at least 2 months. No such antibody response could be detected in monkeys infected with *C. trachomatis*.

Hematology. Characteristically, the erythrocyte sedimentation rate and the blood leukocyte count showed only moderate changes during the investigation period in monkeys infected with either *M. hominis* or *C. trachomatis*.

Controls. The monkeys included as controls presented no signs of inflammation in the genital tract or elsewhere during the investigation period. The body temperature and leukocyte counts remained unchanged from preinoculation values. No antibodies developed against the organisms in any of the monkeys.

Discussion

Nontuberculous salpingitis is, in the vast majority of cases, caused by an infection ascending from the lower genital tract. In principle, two types of such infections are known.[13] In the "gonococcal" type of salpingitis, the organisms are believed to reach the tubal epithelium from the cervix via the uterine mucosa by canalicular spread. The organisms may attack the tubal epithelium, producing a superficial inflammation that may lead to tubal occlusion. The parametria usually are not affected. In the "nongonococcal" type of salpingitis, the organisms primarily invade the genital organs through lesions in the cervical epithelium or the uterine endometrium and spread via blood vessels and lymphatics to the parametria and possibly to the subserosa of the uterine tubes. However, the tubal epithelium usually is unaffected. In the very acute stage, the tubes may be swollen and hyperemic.

The present study shows that in monkeys *M. hominis* may spread via lymphatics and blood vessels to the parametria, producing parametritis and inflammatory changes of the outer layer of the uterine tubes. However, *C. trachomatis* may spread from the cervix canalicularly via the uterine mucosa, producing inflammatory reaction in the tubes with partial destruction of the tubal epithelium.

The possible pathogenicity of *M. fermentans*[9] and *Ureaplasma urealyticum*[6] in the genital tract of female grivet monkeys has been investigated in previous studies. *M. fermentans* was found to produce inflammation in the parametria by lymphogenous/hematogenous spread from the cavum uteri or the cervical epithelium. The appearance of the inflammatory reaction, both grossly and microscopically, resembled that of the lesions provoked by *M. hominis*, although the inflammation was less pronounced. From a clinical point of view, the parametritis provoked by *M. fermentans* is less important than the *M. hominis*–produced inflammation because *M. fermentans* seldom inhabits the genital tract. Attempts at producing salpingitis experimentally with *U. urealyticum* in grivet monkeys have been unsuccessful.

In some of the monkeys with chlamydia salpingitis the tubal epithelium showed inflammatory alterations 2 months after the inoculation, i.e., thickening and adhesions of mucosal folds and reduction of the diameter of the lumen. Such changes may impede transport of a fertilized ovum and thereby impair fertility and even cause sterility. They may also increase the risk of ectopic pregnancy.

In monkeys with upper genital tract infection caused by *M. hominis* or *C. trachomatis*, a significant rise in titer to the organism was present. In a few studies concerning women with acute salpingitis, such a change in serum antibodies to these organisms has been demonstrated. Eschenbach and co-workers[1] showed seroconversion in 15 of 74 women with pelvic inflammatory disease, but they recovered chlamydiae from the peritoneal fluid of only one patient. In a recent investigation[11] we found that 78 of 160 patients with acute salpingitis harbored *M. hominis* in the cervix. Of these 160 patients 44 (28%) presented a significant change in IHA antibodies to *M. hominis* during the course of the disease. This seems to indicate that *M. hominis* is involved in upper genital tract infection in about one fourth of all cases of acute salpingitis. Another study[12] describes the histologic findings of the fallopian tubes in two patients with salpingitis provoked by *C. trachomatis*. The histologic changes in the tubes are very similar to those found in the monkeys with experimentally induced chlamydia salpingitis.

Both the pathologic changes and the mode of spread of the experimentally induced upper genital tract infection by *M. hominis* in grivet monkeys closely resemble those considered characteristic of "nongonococcal" adnexal inflammation in man, while the experimentally induced *C. trachomatis* salpingitis resembles the "gonococcal" type of salpingitis of women.

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In vitro modeling of acute salpingitis caused by *Neisseria gonorrhoeae*

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Normal human fallopian tube organ culture was used as an in vitro model to study *Neisseria gonorrhoeae* infections. The effects of various gonococcal colony phenotypes on morphology of the epithelium were studied by scanning electron microscopy. After 30 minutes' incubation, there was striking attachment of piliated transparent phenotypes to the epithelium; however, there was no obvious pathology. After 24 hours' incubation, there were microcolony formation, slight swelling and hyperplasia of the mucosa, and occasional focal necrosis and sloughing of ciliated cells. Tissue from acute salpingitis showed widespread destruction of mucosa, hyperplasia, and crypt formation. Duplication of these findings in vitro may require longer incubation and the addition of other host factors. (Am. J. Obstet. Gynecol. 138:996, 1980.)

An understanding of the pathologic process involved in the development of acute gonococcal salpingitis is dependent upon an understanding of the virulence characteristics of *Neisseria gonorrhoeae* and the host factors that modify or enhance infection. It has been shown that colony phenotype influences virulence.[1] Opaque colonial variants have been isolated from men with gonococcal urethritis.[2] Although this colony type presumably is transmitted routinely to women and predominates in cervical isolates taken at the middle of the menstrual cycle,[3] it is the transparent variant that is recovered from cervical specimens at the time of menstruation and from fallopian tube exudates in cases of salpingitis.[3, 4] In addition, attachment is believed to play a role in pathogenesis, and piliated *N. gonorrhoeae* attaches in high numbers to human cells in vitro.[5-7]

In this study, the influence of gonococcal piliation and colony opacity/transparency on human explant fallopian tube tissue was examined by scanning electron microscopy. Then the changes were compared with changes in fallopian tube tissue from a patient with active salpingitis.

Materials and methods

Organ culture. Normal human fallopian tube organ culture was used as an in vitro model to study interactions and morphologic changes associated with *N. gonorrhoeae* infections. Normal human fallopian tubes were removed from women undergoing informed, elective tubal ligations. The tissues were immediately processed and maintained in standard tissue culture medium as described previously[8] but with slight modifications.[9] The tissues were used within 24 hours and rinsed thoroughly with antibiotic-free medium before embedding in agar to expose the epithelial surface only. An attempt was made to determine early morphologic and potential pathologic changes of the mucosa during infection by varying the incubation times of the tissue with gonococci.

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Supported by research grants AI 15642 and HD 13462 from the National Institutes of Health.

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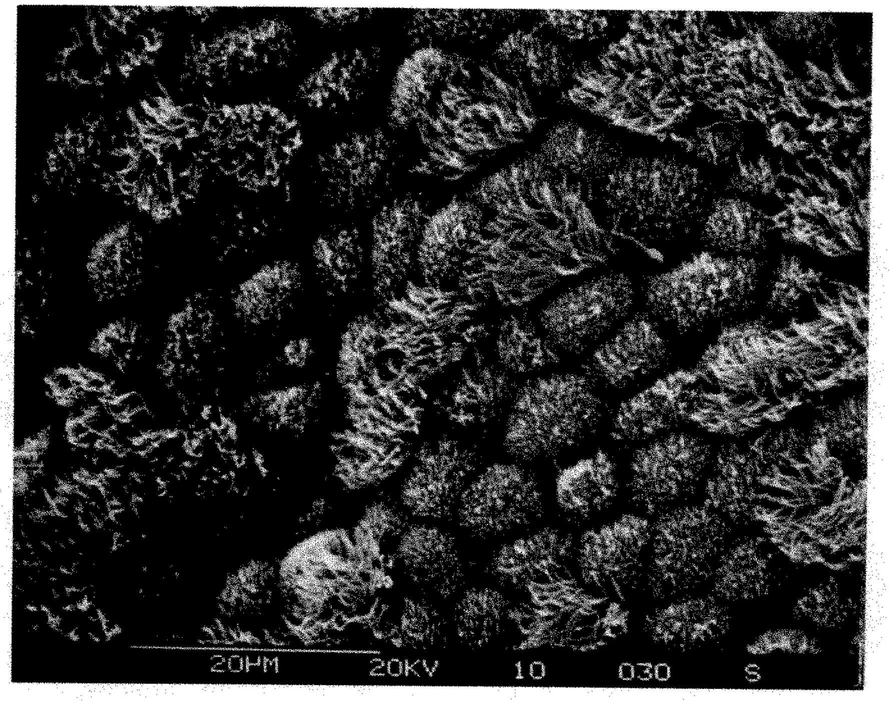

Fig. 1. Scanning electron micrograph of normal human fallopian tube epithelium. The epithelium is composed of a regular layer of nonciliated cells interspersed with ciliated cells. (Magnification ×1,650; bar equals 20 μm.)

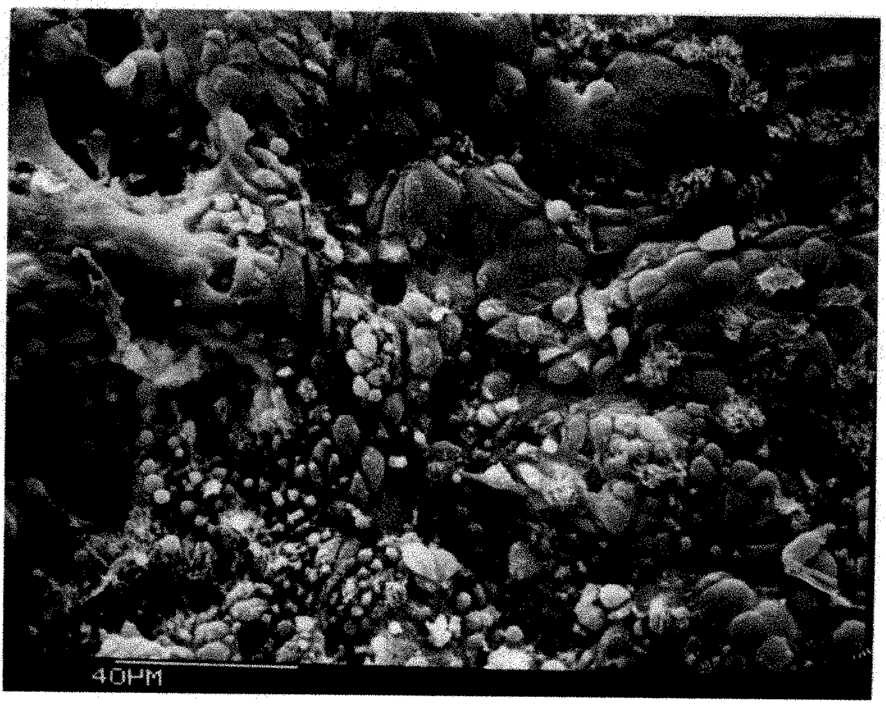

Fig. 2. Scanning electron micrograph of human fallopian tube isthmus from a case of acute salpingitis. The epithelium is irregular, hyperplastic, and disorganized. (Magnification ×625; bar equals 40 μm.)

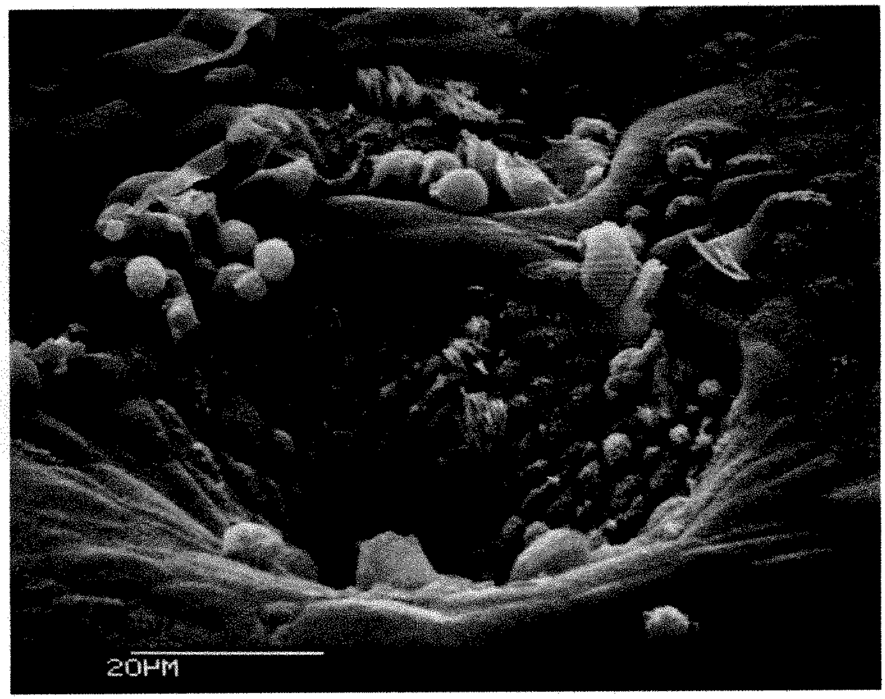

Fig. 3. Scanning electron micrograph of human fallopian tube from a case of acute salpingitis. The cause of the invaginations and crypt-like formations in the epithelium is unknown. (Magification ×1,250; bar equals 20 μm.)

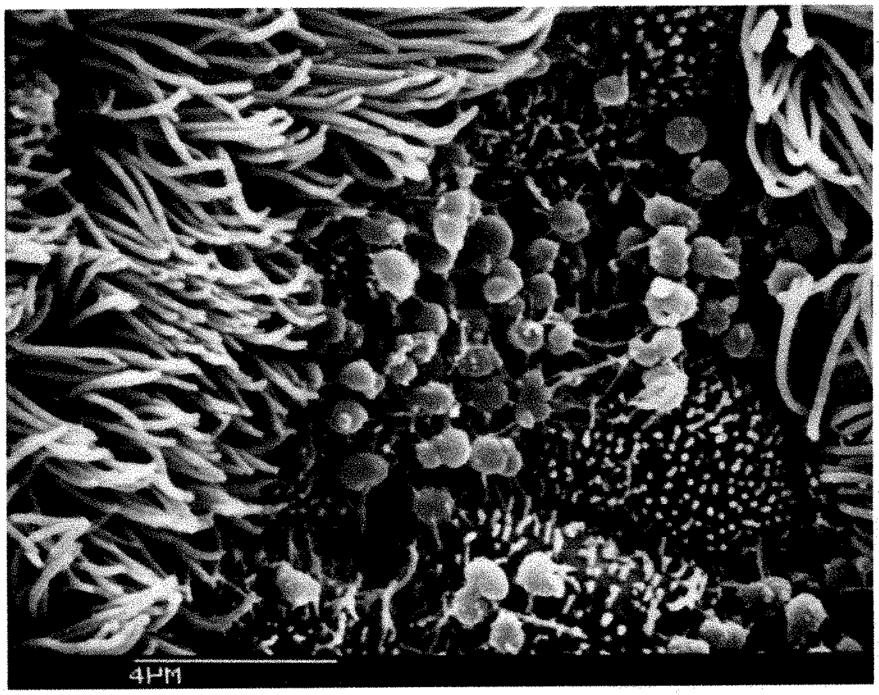

Fig. 4. Scanning electron micrograph of normal human fallopian tube incubated with *Neisseria gonorrhoeae* (P+ +Tr phenotype) for 30 minutes. Attachment of the gonococci was to tips of microvilli of nonciliated, columnar epithelial cells. (Magnification ×6,750; bar equals 4 μm.)

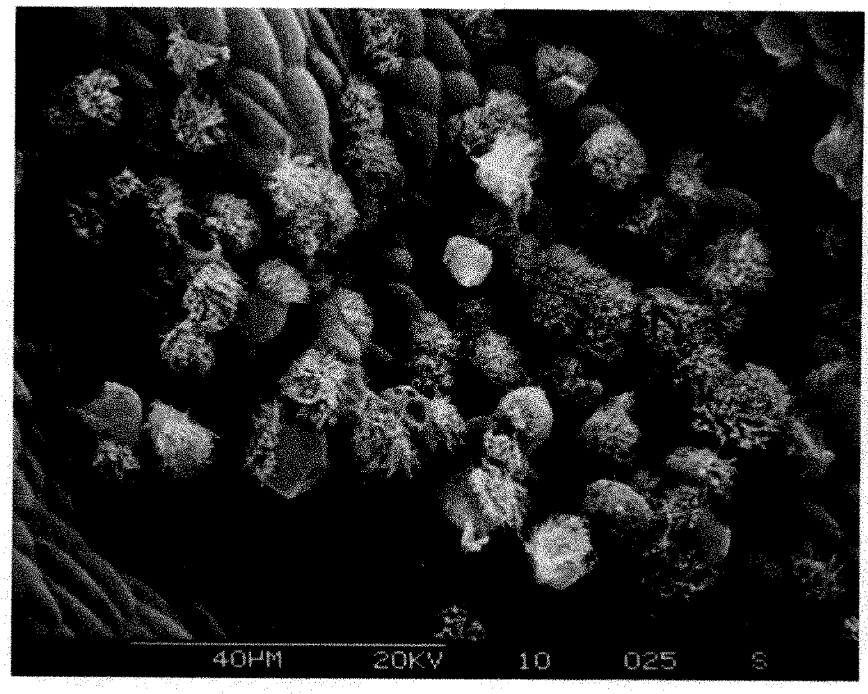

Fig. 5. Scanning electron micrograph of human fallopian tube incubated 24 hours with *Neisseria gonorrhoeae*. There were areas of focal necrosis and sloughing of ciliated cells. (Magnification × 1,050; bar equals 40 μm.)

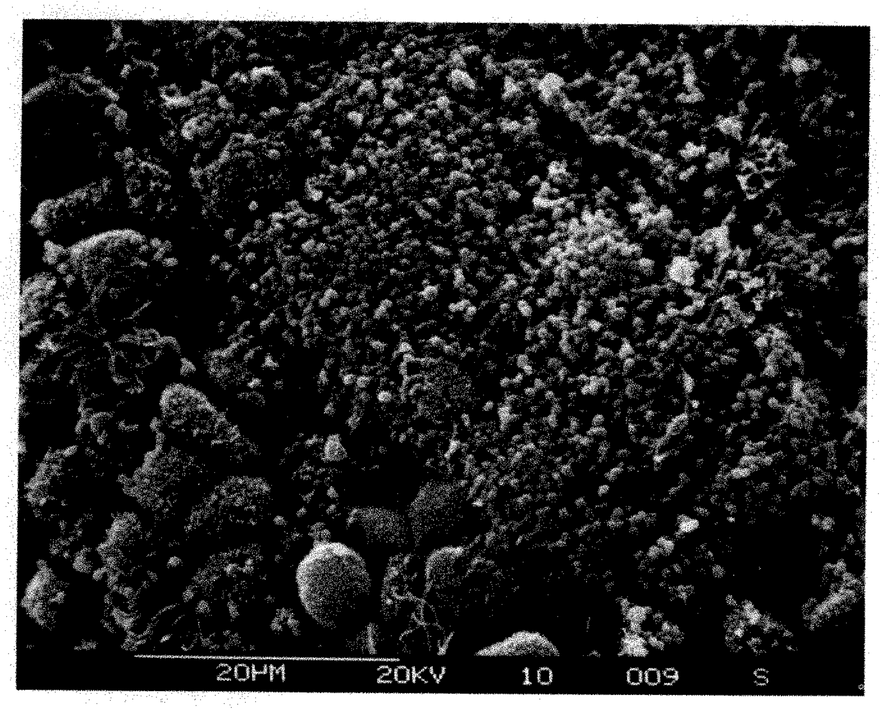

Fig. 6. Scanning electron micrograph of human fallopian tube in organ culture infected with *Neisseria gonorrhoeae* 24 hours previously. Microcolony formation associated with slight swelling of surrounding columnar cells is demonstrated. (Magnification × 1,750; bar equals 20 μm.)

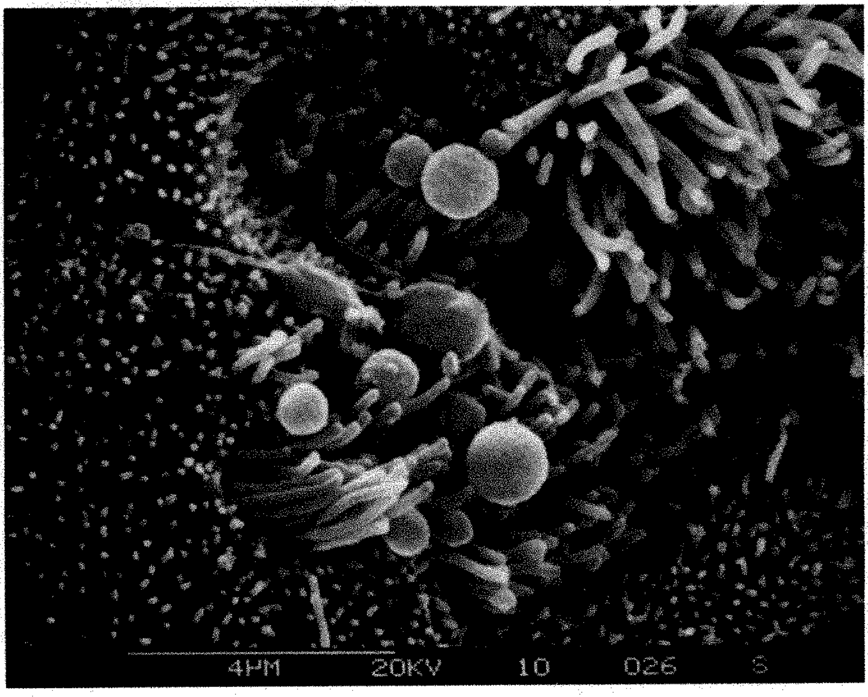

Fig. 7. Scanning electron micrograph of human fallopian tube incubated 24 hours with *Neisseria gonorrhoeae*. Bizzare invaginations of ciliated cells are seen in association with shortening of microvilli and slight swelling of nearby nonciliated cells. (Magnification ×8,250; bar equals 4 μm.)

Organisms. The laboratory strain *Neisseria gonorrhoeae* F62 was used in all studies. Colony phenotypes were passaged daily and maintained on modified GC agar base.[7] Phenotypes were selected according to Swanson[10] and those tested included heavily piliated opaque (P++Op) and isogenic transparent variants (P++Tr). Growths from 16- to 20-hour-old cultures were used to prepare inocula in supplemented Medium 199 (Microbiological Associates, Bethesda, Maryland).[2] An inoculum contained approximately 4×10^7 colony forming units/ml.

Assay. A section of normal fallopian tissue was inoculated with organisms from a particular colony phenotype and incubated for 30 minutes or 24 hours. The tissue was rinsed twice after incubation with Medium 199 to remove nonadherent organisms and was prepared for scanning electron microscopy (SEM).

Scanning electron microscopy. Tissues were fixed immediately in 3% glutaraldehyde (Sigma Chemical Co., St. Louis, Missouri), buffered with 0.1% sodium cacodylate (Sigma), at pH 7.0 for 10 hours at room temperature. Specimens were dehydrated in ascending concentrations of ethanol and dried to critical point with liquid CO_2 as exchange fluid. Specimens were sputter coated with 150 to 200 Å of gold and viewed with a Cambridge S 150 scanning electron microscope.

Salpingitis fallopian tissue. A fallopian tube was recovered from a 19-year-old patient with acute salpingitis. She had a history of one previous episode of gonococcal salpingitis. A culture of fallopian tube exudate obtained by laparoscopy yielded no organisms; persistent severe pain indicated treatment failure, and the left fallopian tube was removed. A small portion of the fallopian tube near the isthmus was prepared for SEM as described above.

Results

Normal fallopian tube tissue. A scanning electron micrograph of normal human fallopian tube epithelium maintained in organ culture for 24 hours is seen in Fig. 1. There were ciliated cells interspersed among nonciliated columnar cells and surface microvilli were a prominent feature. The epithelium was regular and fairly ordered; the cells appeared healthy and intact. There were no distinct morphologic changes associated with the incubation of the tissue in organ culture, and the tissue appeared normal by SEM for 24 hours, and grossly for 14 to 21 days.

Salpingitis fallopian tube tissue. The epithelial surface of a fallopian tube recovered from a patient with acute salpingitis is seen in Fig. 2. The epithelium was disorganized, irregular, and hyperplastic. Invaginations were apparent with "bridge-like" epithelial connections. The invaginations appeared to deepen and form crypts along the mucosal surface (Fig. 3). Areas of hyperplasia usually were seen surrounding the crypts, and there also was an apparent lack of cilia on nearby cells.

Organ culture model. Thirty-minute and 24 hour incubations of tissue were tested with different *Neisseria gonorrhoeae* colony phenotypes. At 30 minutes, there were no distinct morphologic changes of the mucosa associated with the presence of the gonococcus of either the P++Op or the P++Tr phenotype. However, the most striking observation was the enhanced attachment of the piliated, transparent phenotypes to the microvilli of the nonciliated cells (Fig. 4). Ten- to 100-fold more organisms from P++Tr colony phenotypes attached to human fallopian tube epithelium compared to organisms from P++Op colony phenotypes.

In one set of experiments, gonococci were allowed to attach to fallopian tube epithelium for 4 hours, and then nonadherent organisms were removed by rinsing with fresh medium. The tissue was returned to fresh organ culture medium and reincubated. At 24 hours, only subtle yet distinct morphologic changes could be discerned. For tissues incubated with gonococci from either transparent or opaque colonies, there were areas of focal necrosis and sloughing of ciliated cells, and slight hyperplasia (Fig. 5). The formation of gonococcal microcolonies could be observed on the tissue inoculated with organisms from P++Tr pheonotypes (Fig. 6). Surrounding the microcolonies, areas of slight swelling of the columnar cells were suggested by distinct, separate cell borders. Occasionally, bizzare invaginations of single ciliated cells could be seen (Fig. 7). The cells appeared to be withdrawing beneath the level of the surrounding nonciliated columnar cells. The cause of the phenomenon was not discernible. In general, only minimal correlations could be drawn between the morphology of the 24 hour in vitro model and the pathology of the salpingitis tissue.

Discussion

The acute inflammatory process of salpingitis often is associated with serious, chronic sequelae.[10] Involuntary infertility is one of these sequelae, and it presumably is due to scarring, fibrosis, and occlusion of the fallopian tubes.[11] The SEM of salpingitis tissue presented here dramatically demonstrates the destruction of the mucosa and its lack of structural integrity associated with this inflammatory process. There were hyperplasia, sloughing of ciliated cells, and inflammatory exudate present on the epithelium. The presence of invagination and crypt formation in the diseased tissue may provide an explanation for the increased risk of ectopic pregnancy associated with repeated episodes of salpingitis. In this patient, we were unable to culture any organisms from fallopian tube exudate obtained by laparoscopy, and the scanning electron micrographs revealed no potential etiologic agents. Thus, it was not clear what agent(s) caused the destruction of the mucosa.

An interesting observation is that colony phenotypes of *N. gonorrhoeae* recovered from fallopian tube exudate are predominantly transparent[2] and that, in the fallopian tube organ culture model, the gonococci from piliated, transparent colonies are the most adherent to the epithelium.[9] This suggests that gonococci from the transparent phenotypes may be more significant in the invasive process of salpingitis than organisms from opaque phenotypes The ability of *N. gonorrhoeae* to vary its phenotype from opaque to transparent has been described for cervical isolates,[7] and may be important in selection of the invasive form. The environmental and host factors that control the opaque/transparent shift are not known. Further studies are warranted to elucidate the host factors that promote the in vivo expression of transparent phenotype.

Attachment appears to be an important event in pathogenesis. At 30 minutes' incubation, numerous organisms from P++Tr colony phenotypes could be seen attached to microvilli. Organisms from P++Op colonies also attached, but ten- to 100-fold less frequently. A factor or surface property, other than pili, of the transparent phenotypes may be responsible for enhanced attachment of this phenotype to fallopian tube epithelium. The nature of the factor(s) is not known.

Finally, the initial investigations of pathologic changes associated with the infection of human organ culture have produced data showing that at 24 hours only slight hyperplasia and areas of focal necrosis were seen. Crypt formation and invaginations of the epithelium were not observed. To produce these results in the organ culture model may require longer periods of incubation and the addition of other host factors such as serum components, lymphocytes, and polymorphonuclear cells.

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Host defenses in acute pelvic inflammatory disease

I. Bacterial clearance in the murine uterus and oviduct

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Bacterial clearance in the uterine horn and oviduct as a host defense in acute pelvic inflammatory disease was studied in 8- to 12-week-old virgin Balb/c mice. Quantitative cultures of organ homogenates were determined at various intervals following intrauterine injection of a standard inoculum of *E. coli* by micropuncture technique. The effect of uterine horn ligation and of estrus cycle of inoculated mice was evaluated also. Mean bacterial counts in the uterine horn were significantly higher than in the contiguous oviduct ($p < 0.005$), suggesting a barrier function of the uterotubal junction for bacterial passage into the oviduct. Our data suggest a protective role for the uterotubal junction, and demonstrate the influence of uterine obstruction and estrus cycle on bacterial clearance of the oviduct. (AM. J. OBSTET. GYNECOL. 138:1003, 1980.)

THE UTERINE HORN and oviduct of virgin Balb/c mice are normally sterile, suggesting that intact bacterial clearance mechanisms may be operative at these sites. To test this hypothesis, the rate of bacterial clearance in the uterine horn and oviduct of virgin Balb/c mice was determined by quantitative culture of organ homogenates, following intrauterine injection of a standard inoculum of *E. coli* by micropuncture technique. Intrauterine inoculation with bacteria also allows assessment of the role of the uterotubal junction as a barrier to bacterial passage into the contiguous oviduct. The effect of uterine horn obstruction and the influence of the estrus cycle of inoculated mice on bacterial clearance were evaluated also.

Material and methods

Study bacteria. A clinical isolate of *E. coli* (No. 303, serotype 0:75) known to be resistant to mouse serum[1] was used. The organism was isolated on Mueller-Hinton agar and subcultured in defined minimal medium [K_2HPO_4, 14 gm/L; KH_2PO_4, 6 gm/L; $(NH_4)_2SO_4$, 2 gm/L; citric acid, 0.42 gm/L; $MgSO_4$, 0.2 gm/L; glucose, 5 gm/L; and casamino acid, 2.5 gm/L] for 18 hours. Prior to animal inoculation, the organism in late logarithmic phase was centrifuged and resuspended in ice-cold phosphate-buffered saline, pH 7.4, at a final concentration of 10^9 bacteria/ml.

Animal inoculation. Virgin female Balb/c mice, 8 to 12 weeks of age (Canadian Breeding Laboratories, Montreal, Quebec), were anesthetized with pentobarbital (50 mg/kg intraperitoneally), and the left ovary, oviduct, and distal uterine horn were exteriorized aseptically through a lateral flank incision. Under a dissecting microscope, 10 μl of bacterial suspension (approximately 10^7 colony forming units) were injected into the midportion of the exposed uterine horn by use of a siliconized glass micropipet with an external tip diameter of 20 to 25 μ. The incision wound was closed and the animal allowed to recover. The effect of uterine horn obstruction on bacterial clearance was studied by placement of a permanent 7-0 surgical silk ligature around the uterine horn before injection of bacteria

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Supported in part by Medical Research Council of Canada grants DG-188 and DG-189 and by B. C. Health Care Research Foundation grant no. 8(78-3).

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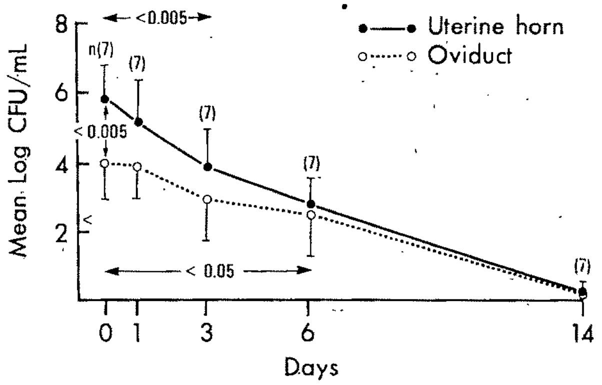

Fig. 1A. Rates of bacterial clearance [mean log colony forming units (CFU)/ml ± SD] from the uterine horn and contiguous oviduct at various intervals following intrauterine inoculation of *E. coli* in estrus *(A)* and metestrus *(B)* virgin Balb/c mice. Number of animals in each group is shown in parentheses.

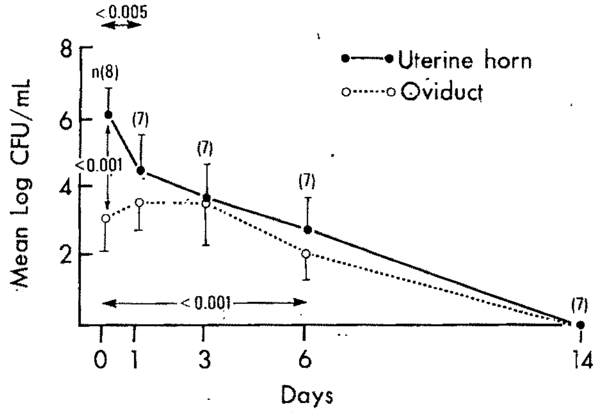

Fig. 1B. See legend to Fig. 1A.

further distally. The estrus cycle was determined by examination of exfoliated vaginal cells stained with methylene blue[2] prior to inoculation and at subsequent sacrifice of each animal (see discussion to follow).

Bacterial quantitation. At various intervals after injection, animals were reanesthetized, and the inoculated uterine horn and adjacent oviduct were removed separately and homogenized in 3 ml physiologic saline (Tri-R tissue homogenizer, Rockville Centre, New York). Quantitative cultures of homogenates were performed in duplicate by serial dilution and pour-plate technique. Bacterial counts were expressed in log counts per milliliter of homogenate.

Table I. Effect of estrus cycle on paired differences in uterine and oviduct bacterial counts immediately following intrauterine inoculation with *E. coli**

	Without uterine obstruction	*With uterine obstruction*
Estrus mice	1.90 ± 0.81 (7)†	4.66 ± 2.31 (17)
Metestrus mice	3.01 ± 1.37 (8)	3.51 ± 1.88 (16)
Rank sum (p)	<0.05	NS‡

*Expressed as mean log difference colony forming units per milliliter ± SD.

†Number of animals in each group in parentheses.

‡Not significant.

Results

The rates of bacterial clearance from the uterine horn and contiguous oviduct at various intervals following intrauterine inoculation are shown in Fig. 1A for estrus mice and in Fig. 1B for metestrus mice. Immediately after intrauterine inoculation of *E. coli*, mean bacterial counts were significantly lower in the adjacent oviduct than the inoculated uterine horn for both estrus and metestrus mice ($p < 0.005$, paired t test, two-tailed). This suggests that the uterotubal junction maintained a definite barrier for bacterial passage into the oviduct immediately after intrauterine inoculation. The mean difference in bacterial counts between the uterine horn and its adjoining oviduct was significantly lower in estrus mice than in metestrus mice in the absence of uterine obstruction ($p < 0.05$, rank sum test, Table I). This suggests that the uterotubal barrier is less effective during estrus than metestrus, although this difference is not observed after inoculation in animals with uterine horn obstruction (Table I). In the uterine horn, mean counts in metestrus mice were significantly reduced as early as 24 hours after inoculation (Fig. 1B), while in estrus mice significant reduction in bacterial counts was delayed until 3 days after inoculation (Fig. 1A). Mean counts in the oviducts of both estrus and metestrus mice were not significantly reduced until 6 days after inoculation. Viable *E. coli* was eliminated completely from both uterine horn and oviduct in almost all animals at 14 days after inoculation.

The patterns of bacterial clearance in the presence of uterine obstruction in mice inoculated during estrus are depicted in Fig. 2A (uterine horn clearance) and Fig. 2B (oviduct clearance), and contrasted with patterns in mice without uterine obstruction. Similar patterns were observed for metestrus mice that are not depicted here. Notably, mean bacterial counts again were significantly lower in the oviduct compared to the uterine horn immediately following intrauterine inoculation ($p < 0.001$, paired t test, two-tailed). This suggests that the uterotubal junction maintained a definite barrier for bacterial passage into the oviduct, even in the presence of uterine obstruction. However, significant reduction of bacterial counts in the obstructed uterine horn was delayed until 6 days compared to 3 days in unobstructed animals (Fig. 2A). Significant viable counts remained in the obstructed uterine horn even at 14 days after inoculation. With uterine horn obstruction, mean bacterial counts in the oviduct significantly *increased* at 3 days after inoculation ($p < 0.01$, unpaired t test, two-tailed, Fig. 2B) in contrast to counts in mice without uterine obstruction which decreased. Bacterial clearance in the oviduct did occur at 6 days after inoculation despite uterine horn obstruction, although significant counts still remained at 14 days. All animals remained clinically well prior to sacrifice, and no evidence of active infection was noted in the pelvic peritoneum or genital tract at harvest.

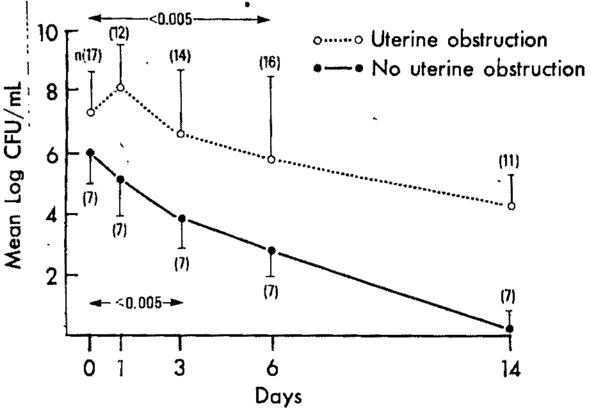

Fig. 2A. Rates of bacterial clearance (mean log CFU/ml ± SD) from the uterine horn *(A)* and contiguous oviduct *(B)* in estrus mice with or without uterine horn obstruction. Number of animals in each group is shown in parentheses.

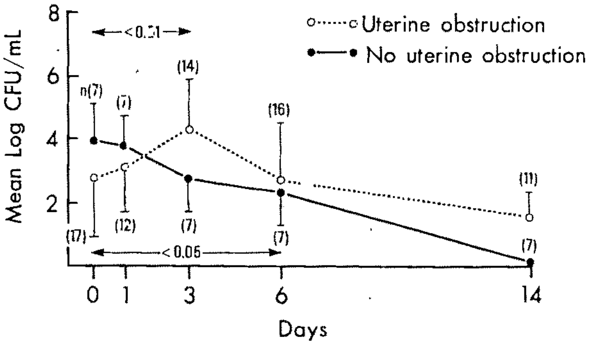

Fig. 2B. See legend to Fig. 2A.

Discussion

The pathogenesis of acute PID is poorly understood, although ascending infection by direct spread of organisms from the cervix is believed to be the common route. Surprisingly, little is known about host defenses against microbial invasion in the human upper genital tract. Since these sites normally are sterile, intact bacterial clearance mechanisms must be operative in the normal host.

Our studies indicate that in virgin Balb/c mice, bacterial clearance occurs both in the uterine horn and the oviduct, although clearance in the latter is delayed. Furthermore, the uterotubal junction appears to have a protective role by serving as a definite barrier for bacterial passage into the oviduct from the adjoining uterine horn. More importantly, these studies demonstrate the influence of the estrus cycle and uterine obstruction on bacterial clearance in the oviduct. Our data also suggest a detrimental effect of the estrus phase on the barrier role of the uterotubal junction. These findings are in keeping with previous observations during insufflation studies that the uterotubal junction is an effective barrier in the mouse[3] and rat,[4] and that the opening pressure may vary with the stage of the estrus cycle.[4] It is of interest that the high incidence of salpingitis in cows has been ascribed to an ineffective barrier function of the uterotubal junction.[5] Further studies should be initiated to elucidate the mechanism of bacterial clearance in the upper genital tract, including the potential role of mucociliary function, oviductal fluid, secretory immunoglobulins, as well as phagocytic and cellular immune systems. Better understanding of the interactions between microbial and host factors is clearly needed in order to provide a more rational and effective approach to prevention and treatment of acute PID.

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CLINICAL SPECTRUM

Differential diagnosis of acute pelvic inflammatory disease

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Laparoscopic investigations have shown that clinical symptons and signs in cases of acute pelvic inflammatory disease (acute salpingitis) show considerable variation and seem to a great extent to be nonspecific. Diagnosis based on clinical criteria alone is, therefore, unacceptably unreliable. Different intrapelvic disorders and acute infections limited to the lower genital tract (LGTI) represent substantial differential diagnostic problems. The routine use of laparoscopy is currently the best method for solving these problems but its broader application is restricted by several factors. Determination of specific genital isoamylases obtained at vaginal puncture of the cul-de-sac seems to represent a promising and specific laboratory test for differentiating between acute PID and LGTI that simulates acute PID. (AM. J. OBSTET. GYNECOL. 138:1006, 1980.)

THE TRADITIONAL view of acute pelvic inflammatory disease maintains that its clinical characteristics are fairly uniform and definable on the basis of history, physical examination, and common laboratory tests. The major differential diagnostic problem is thought to concern the diagnosis of PID vs. acute appendicitis. The "classical" set of clinical criteria for PID, listed in Table I, is well known and has proved to be "durable" through the years. However, the criteria may be criticized on the basis of their reversed logic, in which the disorder has been made to fit the criteria established for it and not vice versa.

During the 1960s, a series of systematic laparoscopic studies on acute PID were conducted by Falk[1] and by Weström and me,[2, 3] at the University Departments of Gynecology in Lund and Malmö, Sweden. These studies resulted in a considerably revised view of both the clinical manifestations of acute PID and the problems of differential diagnosis. These investigations were part of an extensive project, initiated by Sjövall, head of the Department in Lund; the purpose of this project was to develop laparoscopy as a simple and routine procedure to be fully integrated into gynecologic procedures as a whole. This policy has been accepted subsequently by every gynecology department in Sweden.

Our most extensive and detailed study included all cases of acute salpingitis visually verified at the Department in Lund during an 8 year period from 1960 through 1967.[3] This article is based primarily on that study.

The basic design of the study involved the evaluation of the effectiveness of classical criteria in diagnosing acute PID as compared with the results of laparoscopy. The medical setting within which the study took place provided the opportunity for detecting the existence of all cases of acute PID, even including those originally receiving a different, tentative diagnosis on clinical grounds.

For special purposes of the study the most probable diagnosis was established through careful examination by experienced clinicians and was formally registered prior to laparoscopy, which took place within 24 hours and before any treatment was started.

Table II summarizes the data. Among the 814 cases

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Table I. "Classical" clinical criteria of acute PID

1. Acute pelvic pain of a few days' duration
2. Fever of 38° to 39° C
3. Tender adnexal swelling or mass
4. ESR
   WBC count } elevated in blood

Table II. Comparison of laparoscopic findings in patients with presumptive clinical diagnosis of PID

		Visual diagnosis (no.)		
Presumptive clinical diagnosis	*No.*	*Acute PID*	*Other pelvic disorders*	*No pelvic pathology*
Acute PID	814	532	98	184
Other than acute PID	91	91		
Total		623		

of suspected acute PID, laparoscopy results were true positive for 532 of these. The diagnostic accuracy of the clinical criteria was 65%.

The overall accuracy rate for diagnosing acute PID on the basis of clinical criteria did not improve during the 8 year course of the study, despite very close attention paid to those patients. Subsequent investigations done by us and others using a similar study design also have shown that it seems almost impossible to exceed the 65% accuracy level mentioned. With the exception of the figure of 89%, recently reported by Sweet and his associates[4] for a small and selected series of 29 cases, the clinical diagnostic accuracy generally has been found to be lower than 65%, and even as low as about 50%.[5-11]

What are the reasons for this unacceptably low diagnostic accuracy when *using clinical criteria,* and what other disorders constitute the main differential diagnostic errors? As seen in Table II, three groups of patients could be distinguished.

Group 1—false positive PID but other pelvic disorders: There were 98 (12%) patients with clinically suspected PID in whom laparoscopy revealed other pelvic disorders.

Group 2—false negative PID: There were 91 cases in which acute PID was not suspected clinically but laparoscopy or occasionally laparotomy demonstrated the true diagnosis. These patients constituted 15% of the total 623 PID cases.

Group 3—false positive PID and no pelvic pathology: There was a large group of 184 patients (23%) in whom the clinical assumption of PID was not substantiated upon laparoscopy and absolutely no intrapelvic pathology was found.

Table III. Laparoscopic findings in patients with false positive clinical diagnosis of acute PID but with pelvic disorders other than PID

Laparoscopic findings	*No.*
Acute appendicitis	24
Endometriosis	16
Corpus luteum bleeding	12
Ectopic pregnancy	11
Pelvic adhesions only	7
Benign ovarian tumor	7
Chronic salpingitis	6
Miscellaneous	15
Total	98

Table IV. Laparoscopy/laparotomy diagnoses in patients with false negative clinical diagnosis of acute PID by laparoscopy

Clinical diagnosis	*Visual diagnosis: acute PID (no.)*
Ovarian tumor	20
Acute appendicitis	18
Ectopic pregnancy	16
Chronic salpingitis	10
Acute peritonitis	6
Endometriosis	5
Uterine myoma	5
Uncharacteristic pelvic pain	5
Miscellaneous	6
Total	91

The details regarding group 1 are listed in Table III. The main differential diagnostic errors involved acute appendicitis, pelvic endometriosis, and intrapelvic bleeding due to various causes (ectopic pregnancy, corpus luteum hemorrhage, retrograde menstruation). Also, sequelae of previous acute PID, i.e., chronic salpingitis and adhesions, occasionally stimulate acute PID, as did benign ovarian tumors.

Table IV illustrates the opposite diagnostic discrepancy (group 2). Again, acute appendicitis, intrapelvic bleeding, chronic inflammatory conditions, and endometriosis constituted the major differential diagnostic difficulties. However, acute PID frequently was mistaken for an ovarian tumor on clinical grounds. These two groups represent diagnostic mistakes which, if not corrected by laparoscopy (or laparotomy) may involve serious consequences for the patients.

Group 3—those cases showing no intrapelvic pathology at all—was the largest and perhaps most surprising group, consisting of about one fourth of all patients suspected of having acute PID. In order to evaluate the effectiveness of the classical clinical criteria in identifying acute PID, the frequency of these symptoms and

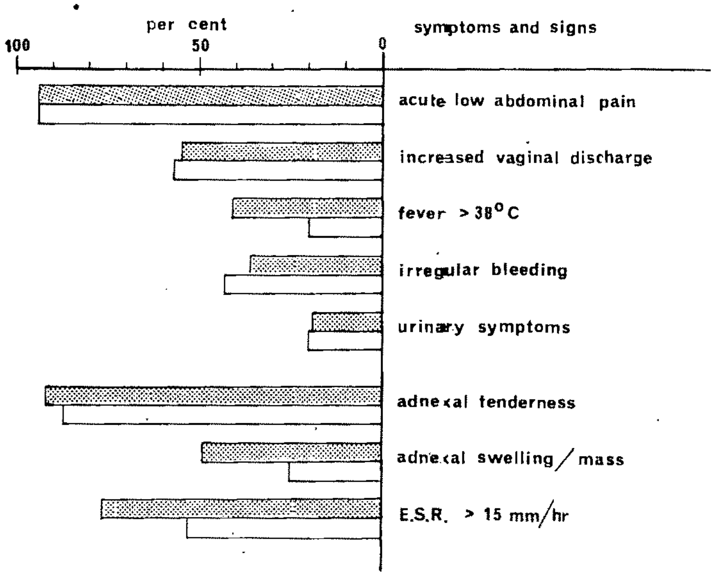

Fig. 1. Comparison of frequency of symptoms, signs, and laboratory findings between patients with acute PID *(dotted bars)* and suspected PID without pelvic pathology *(open bars)* by laparoscopic examination.

Table V. Duration of pain in patients with visual confirmation of acute PID by laparoscopy

	Days					
	0-3	*4-6*	*7-9*	*10-12*	*13-15*	*>16*
% in study by Falk[1] (n = 283)	29	23	18	8	5	17
% in study by Weström[12] et al. (n = 623)	31.5	15	19	8	10	16.5

signs was compared for the 623 true positive vs. the 184 false positive cases involving no pelvic pathology (Fig. 1). By and large, the traditional clinical criteria for PID were found to exist as frequently in the group with no intrapelvic pathology as in the true PID group. A statistically significant increase of symptoms in the PID group was found only for fever, palpable adnexal swelling or mass, and elevated erythrocyte sedimentation rate (ESR). However, as evident, the frequency of these characteristics in the "no intrapelvic pathology" group was too high to allow these variables any differential diagnostic value on a specific-case level. These findings also illustrate the often unrecognized fact that bimanual examination performed even by experienced clinicians may be quite unreliable.

The rather high frequency of menstrual irregularities in acute PID should be noted. As seen in Fig. 1, this symptom was found in approximately 40% of cases and may contribute to the fact, mentioned above, that clinically PID often is mistaken for ectopic pregnancy, and vice versa.

As also seen in Fig. 1, not even the presence of acute lower abdominal pain is a necessary feature of the clinical picture in acute PID. In fact, the duration of pain prior to admission may vary greatly, as has been shown by Falk[1] and Weström[12] in separate series (Table V).

It would seem reasonable to assume that patients with true acute PID to some extent might be differentiated clinically from normal subjects clinically suspected of having PID, by considering the *number* of symptoms and signs presented by the individual patient. As seen in Fig. 2, an almost linear correlation was found between the number of clinical variables and the reliability of the clinical diagnosis.[3] However, this fact does not contribute very much to the clinical diagnostic efficiency, as patients with many symptoms and signs represent but a small fraction of the clientele, and patients with few clinical variables often have acute salpingitis.

Furthermore, it should be emphasized that the correlation between the number and intensity of symptoms, on the one hand, and the severity of the tubal inflammation, on the other, may show considerable variation for different conditions of various microbiologic etiologies. Gonococcal salpingitis, even when mild and with a favorable prognosis, often presents with multiple symptoms, whereas acute PID caused by other microorganisms (particularly chlamydia) may evidence few symptoms despite the presence of a severe inflammatory reaction of the fallopian tubes.

What are the differential diagnoses in patients clinically suspected of having PID but with normal laparoscopic examinations? Thorough investigation by Weström and Mårdh[13] has shown that in the vast majority of cases (approximately 90%) a lower genital tract infection (LGTI) of different etiology was present and reasonably should account for the clinical symptoms and signs. Those findings are interesting for they indicate that the clinical symptoms and signs recorded in verified cases of acute PID did not have their main origin in the fallopian tubes per se, but in anatomic structures outside the pelvic cavity.

Thus, two large groups of disorders which contribute to the substantial difficulties in the differential diagnosis of acute PID could be defined: (1) other pathologic intrapelvic conditions of an acute (and also nonacute) nature and (2) LGTI of different microbiologic etiology.

To complete the list, a third group should be added: the Fitz-Hugh–Curtis syndrome or perihepatitis associated with PID. The syndrome is described as a condition in which an exudative inflammation appears in the liver capsule, secondary to the intrapelvic infection, and later leaving violin-string adhesions. As the symptoms may simulate acute cholecystitis, the primary presence of the acute PID might be concealed. Different authors report the incidence of perihepatitis to be within the range of 1% to 30% of all cases of acute PID, particularly those of gonococcal etiology.[14, 15] The true incidence is difficult to evaluate, however, as very few reported cases were verified by laparoscopy or laparotomy.[16, 17] According to the experience gained from the extensive use of routine laparoscopy in Sweden, the syndrome is uncommon.

Thus the current view of acute PID, based on systematic laparoscopic investigations, differs in many respects from the "classical" concept. The overall result of our investigations is that the accuracy of diagnosing acute PID on the basis of clinical characteristics is unacceptably low. The major clinical concern, therefore, is how to improve the accuracy of diagnosis. It would seem that the problem may be solved in three ways: (1) intensify attempts to extend current knowledge of diagnostic and differential diagnostic facts regarding acute PID, (2) extend use of diagnostic laparoscopy, and (3) intensify research on other, specific diagnostic means. The first point is challenging and is included because specific interest and detailed knowledge as to differential diagnostic patterns of acute PID still differ among all those medical units that are confronted daily with such patients. Any steps taken to extend knowledge and attention in this field will result in diagnostic progress.

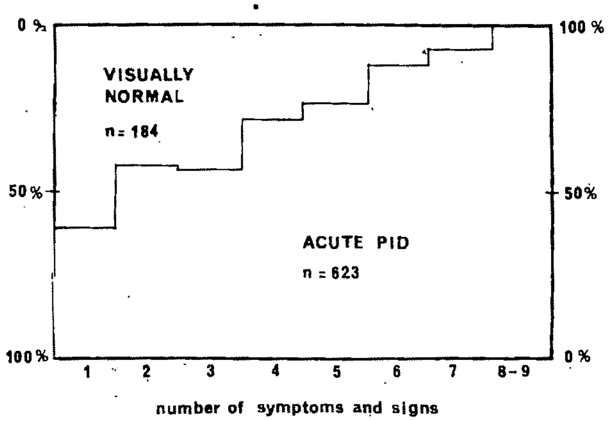

Fig. 2. Percentage distribution of acute PID cases and suspected but visually normal cases with regard to nine clinical criteria.

Table VI. Laparoscopic criteria of acute PID

Minimum criteria:
1. Erythema of fallopian tube
2. Edema and swelling of fallopian tube
3. Seropurulent exudate from fimbriated end or on serosal surface of fallopian tube

Scoring:
Mild: minimum criteria; tubes freely movable and patent
Moderate: minimum criteria more marked; tubes not freely movable; patency uncertain
Severe: inflammatory mass

No doubt, the routine use of laparoscopy is, for the present, the most reliable procedure in the diagnosis and differential diagnosis of acute salpingitis. According to Swedish experience,[2, 3, 12] a correct diagnosis is easily obtainable in 95% of cases. In approximately 4%, pelvic adhesions or borderline criteria of tubal inflammation may render the visual assessment of the tubes more difficult, and in 1%, the methods fail for diverse technical reasons (multiple adhesions, distended intestinal loops, obesity, etc.). Strict criteria for the visual evaluation of PID are necessary (Table VI), especially for a uniform scoring of the inflammatory conditions. The complication rate of laparoscopy is generally low, and in PID series it is almost nil owing to the fact that those patients are mainly young and in a good general condition. In our laparoscopy series, insignificant complications were encountered in two to three per 1,000 cases (extraperitoneal CO_2 insufflation, hematoma of the abdominal wall); in no instance did any significant complication occur.

Laparoscopy may fail in cases of acute salpingitis restricted to the endosalpinx. The extent of this source of error is not known exactly, but it would seem very small judged on the basis of the thorough follow-up studies by Weström[12] of all suspected but visually normal cases

Table VII. Tentative laboratory tests in diagnosing acute PID

1. Microbiologic determinations
2. WBC in vaginal wet smear
3. "Acute-phase" plasma protein determination
4. Specific genital isoamylases in peritoneal fluid
5. WBC count in peritoneal fluid

Table VIII. Peritoneal fluid tests (according to Weström and associates[12, 13])

	Acute PID	*Visually normal*
Quotient between isoamylase activity in peritoneal fluid and serum	<1.5	>1.5
WBC count in peritoneal fluid (cells/L)	$31\text{-}37 \times 10^9$	$0.3\text{-}1.3 \times 10^9$

of PID. The frequency of failure may be reduced further by the consistent use of a manipulation cannula, inserted next to the laparoscope. Thus the tubes can be maneuvered and slightly squeezed for any small amount of exudate.

The main obstacles to the increased use of diagnostic laparoscopy are organizational and economical but there is also personal reluctance to use the method at many institutions. There is, therefore, no doubt an urgent need for alternative and specific diagnostic methods. Different laboratory tests have been considered thus far and are listed in Table VII.

Bacteriologic (and serologic) determinations do not add to the diagnostic or differential diagnostic possibilities in acute PID.

As shown by Weström[12] in a series of 327 verified cases of acute PID and 105 suspected but laparoscopically normal cases, the complete absence of *white blood cells (WBCs) in vaginal wet smears* is not consistent with acute PID—the presence of WBCs has no differential diagnostic value.

The reaction of certain *inflammatory plasma proteins* (the "acute-phase reactants" orosomucoid, antichymotrypsin, and C-reactive protein) in cases of acute PID and suspected acute PID with normal tubes was independently investigated by Jacobson and colleagues[8] and Weström[12] in two series of 72 and 65 patients, respectively. Increased plasma protein concentrations were observed in approximately 75% of the PID cases and in approximately 21% of the patients with LGTI. The test is thus statistically somewhat better than ESR in differentiating those two conditions but it is not specific for acute salpingitis.

Determination of *specific genital isoamylases* in peritoneal fluid, obtained by vaginal puncture of the Douglas pouch, is a new and promising method, recently initiated by Weström, Skude, and Mårdh.[18] The genital isoamylases, which are electrophoretically separable from pancreatic and salivary isoamylases, are mainly responsible for the amylase activity in peritoneal fluid but are not detectable in peripheral blood. In tubal inflammation the specific amylases disappear from the peritoneal fluid. The decrease was found to be proportional to the severity of the tubal inflammation and not related to patency or closure of the tubal ostia. In laparoscopically normal cases of LGTI, the amylase activity in peritoneal fluid was normal. The quotient between nonpancreatic amylase activity in peritoneal fluid and in serum was found to be >1.5 in nonsalpingitis and <1.5 in acute salpingitis cases. This laboratory test is the most specific for acute PID found thus far (Table VIII).

Furthermore, Weström and Mårdh[13] showed that the *WBC count in peritoneal fluid,* obtained by cul-de-sac puncture, differed significantly between verified cases of acute PID and clinically suspected, but visually normal, cases (Table VIII). Vaginal puncture and determination of specific isoamylases and WBC count may thus become an alternative method in differentiating between acute PID and LGTI, clinically suspected to be acute PID, and might perhaps be applicable as an office procedure. However, the methods have to be evaluated further.

In summary, we conclude, from our data obtained by systematic laparoscopic assessment, that diagnosis of acute PID based solely on clinical symptoms and signs is, on the average, about 60% to 65% accurate. The wide range of frequent diagnostic mistakes includes intra- and extrapelvic disorders of both a serious and benign nature. With any given patient, one is therefore very seldom, if ever, justified in feeling confident about the diagnosis of acute PID made on the basis of clinical criteria. One can only feel more or less uncertain. Unidentified and untreated acute PID is no doubt an important cause of chronic pelvic inflammatory disorders and infertility. Great effort therefore has to be made to improve accuracy in diagnosing acute PID by increased application of specific diagnostic methods.

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Laparoscopy in the diagnosis and management of patients with suspected salpingo-oophoritis

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There is a potential role for laparoscopy in the management of patients with suspected salpingo-oophoritis. It would be helpful in establishing the diagnosis in patients who are admitted with a clinical diagnosis of salpingo-oophoritis. Laparoscopy also would be beneficial in the long-term evaluation of fertility of patients treated for salpingo-oophoritis. Finally, this technique will delineate the microbiologic details of patients with endosalpingitis. How frequently laparoscopy should be employed will be dependent upon findings from prospective studies. (AM. J. OBSTET. GYNECOL. 138:1012, 1980.)

THERE IS A reluctance among American physicians to utilize invasive operative techniques in the diagnosis and management of patients with suspected salpingo-oophoritis. Conversations with many physician groups in this country show a majority do not even employ needle culdocentesis in the evaluation of nonpregnant patients with pelvic infection and there is even rarer use of laparoscopy for the detailed analysis of such cases. There are a number of reasons for this practice pattern. Most of these patients are uncomfortable when first seen by the physician and there is reluctance to perform any diagnostic procedure that would increase discomfort. Probably more importantly, most of these patients in the United States are treated as outpatients, and physicians want to avoid any operative procedure that would require admission to the hospital. Finally, there is the common physician assumption that this complicated diagnostic procedure is not necessary because antibiotics nearly always cure salpingitis, which is not life-threatening.

Any thought to modify this American pattern of rare use of the laparoscope in the diagnosis and management of patients with salpingo-oophoritis must assess the risk/benefit ratio of such an alteration. Is there a risk to the patient if laparoscopy is to be employed more widely? Of course there is. Most investigators favor the use of general anesthesia when doing laparoscopy in patients with suspected salpingitis and it has been estimated that there will be 4.6 major complications per 1,000 cases, with a mortality rate of 5.2 per 100,000 cases.[1] Reports to date on the evaluation of salpingitis with laparoscopy have not detailed unfortunate outcomes, but these poor end results should be expected if more and more of these diagnostic procedures are done. In addition to these major complications, laparoscopy may be impossible to perform in some women. To balance these recognizable risks, there should be documented patient benefit from the widespread use of this diagnostic technique. The potential benefits can be categorized into three groups. The first is immediate diagnosis. Is there any evidence that the current clinical criteria for the diagnosis of salpingitis are always accurate? If this is frequently an incorrect diagnosis, laparoscopy would permit more accurate therapy for individual patients. The second potential benefit is in the area of prognostic studies. Is there evidence that laparoscopy would aid in the evaluation of the long-term fertility of women exposed to various methods of therapy for their salpingitis? If so, this could result in better therapeutic strategies in such patients in the future. The third potential benefit is better microbiologic understanding of the disease. Is there any evidence that the microbiologic data obtained at the time of laparoscopy will change our understanding of the pathophysiology of this disease?

Immediate diagnosis

There is good evidence that the current clinical criteria for the diagnosis of salpingo-oophoritis are not 100% accurate, and that they can be improved by the use of laparoscopy. Jacobson and Weström,[2] in 1969, authored a comprehensive evaluation of this subject.

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Table I. Total material: preexploration diagnoses and subsequent visual findings

Preexploration diagnosis	*No.*	*Visual diagnosis (no.)*		
		Acute salpingitis	*Other disorders*	*Normal*
Acute salpingitis	814	532	98	184
Other than acute PID	91	91		
Total	905	623	98	184

Table II. Erroneous preexploration diagnoses of acute salpingitis—visual findings

Visual diagnosis	*No.*
Acute appendicitis	24
Pelvic endometriosis	16
Corpus luteum hematoma	12
Ectopic pregnancy	11
Ovarian tumor	7
Chronic salpingitis	6
Mesenteric lymphadenitis	6
Miscellaneous	16
Total	98

Their criteria for the usual diagnosis of salpingitis depended upon the presence of three signs: (1) pronounced hyperemia of the tubal surface, (2) edema of the tubal wall, and (3) a sticky exudate on the tubal surface and from the fimbriated ends when patent. These criteria have not been accepted universally and the concern has been that all these criteria may not be met in every woman with endosalpingitis. Hans Hirsch* has found hyperemia to be absent at laparoscopy in many women with acute salpingitis, and I fear that some women with early endosalpingitis may not have gross changes on the surface of the tube viewed at laparoscopy. With these potential shortcomings in mind, the clinical results of the Swedish study were astounding.[2] Table I documents the preexploration diagnosis and subsequent visual findings. In only 65% of the patients with suspected salpingitis was this confirmed, and in 12%, other pathologic conditions were found. In 23%, no pelvic pathology was discovered. Table II lists other pathologic findings in patients with suspected salpingitis. Prolonged use of potentially toxic antibiotics should be avoided in such women. In an evaluation of the helpfulness of various symptoms in confirming the clinical diagnosis of salpingitis, only fever and proctitis were associated with salpingitis in a statistically significant number of cases. Table III documents the populations of women with salpingitis and those that had normal visual examinations. There clearly are two aspects of these diagnostic inaccuracies. The first is the overdiagnosis of salpingitis in patients with pelvic pain when other clinical problems are present (Table II). The second is the absence of salpingitis in some women with symptomatology associated with salpingitis. Table IV documents the frequency of objective findings in the salpingitis and visually normal groups. Although statistically significant differences are noted, these findings are not reassuring clinically because of the wide overlaps in the two populations. Difficulties in the clinical diagnosis of salpingitis are not limited to the Swedish studies. Eschenbach and colleagues[3] found 10% of their patients had other diagnoses when laparoscopy was employed. A major clinical problem in the recognition of salpingitis is the lack of fever in many of these women when first evaluated. Over 50% of the women in the Swedish study[2] had fever of less than 38° C while Eschenbach and associates[4] showed fever to be absent frequently in women with salpingitis, particularly when an intrauterine device was present. It is not known how frequently these patients can be identified by needle culdocentesis alone, but laparoscopy would be a definitive diagnostic technique in such women.

The exact role of laparoscopy in the diagnosis of acute salpingitis has not been established. It is clear that clinical errors are made in both directions. Some cases of salpingitis are not diagnosed clinically and some patients with other conditions are thought inaccurately to have salpingitis. The risk/benefit ratio of more widespread use of this procedure has not been established. Since most studies to date have employed general anesthesia in women with suspected salpingitis, it is unlikely that there will be great enthusiasm for the evaluation by laparoscopy of women ordinarily treated as outpatients. This is particularly true of patients with afebrile salpingitis. There may be a wider use of this procedure in women sick enough to be treated as inpatients. Since admission is required and more potent and toxic antibiotics usually are employed, laparoscopy to define the diagnosis and the extent of the disease would be a more acceptable procedure to American physicians. The overall impact on patient care awaits prospective study of a large number of such women.

Prognosis—the impact of therapy upon human childbearing potential

The vast majority of clinical studies of the treatment of women with acute salpingitis have been seriously flawed. In addition to the aforementioned concerns about proper diagnosis, there is a vast gap in our information about the impact of therapy. Most evalua-

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*Hirsch, H.: Personal communication.

Table III. Frequency of various symptoms as reported by the patients in the salpingitis and visually normal groups

	Visual diagnosis				
	Acute salpingitis (623 cases)		*Normal with acute PID clinically (184 cases)*		
Symptoms	*No.*	*%*	*No.*	*%*	*P*
Low abdominal pain	585	93.9	173	94.0	NS*
Increased vaginal discharge	340	54.6	104	56.5	NS
Temperature >38° C or chills	257	41.3	36	19.6	<0.001
Irregular bleeding	221	35.5	79	42.9	NS
Urinary symptoms	116	18.6	37	20.1	NS
Vomiting	64	10.3	17	9.2	NS
Proctitis symptoms	43	6.9	5	2.7	<0.05
Other	17	2.7	7	3.8	NS

*Not significant.

Table IV. Frequency of various objective findings at admission in the salpingitis and the visually normal groups

	Visual diagnosis				
	Acute salpingitis (n = 623)		*Normal with acute PID clinically (n = 184)*		
Clinical findings at admission	*No.*	*%*	*No.*	*%*	*P*
Bimanual examination					
Marked tenderness only	573	92.0	160	87.0	<0.05
Palpable mass or swelling	308	49.4	45	24.5	<0.001
Erythrocyte sedimentation rate ≥15 mm/hr	473	75.9	97	52.7	<0.001
Abnormal vaginal discharge	394	63.2	74	40.2	<0.001
Fever (>38° C)	205	32.9	26	14.1	<0.001

Table V. Fertility after salpingitis*

No. of episodes	*Occlusion of tubes*
1	12.8%
2	35.5%
≥3	75%

*Data from Westrom, L.: Am. J. Obstet. Gynecol. **121:** 707, 1975.

tions of various methods of antibiotic therapy focus upon the immediate clinical response.[5] A number of measures have been employed. How rapidly do the patients become afebrile after the initiation of therapy? This may be gauged by the number of days required for a return to a normal temperature or by the quantity of fever as measured by the fever index in degree-hours.[6] Another evaluation is the loss of lower abdominal and pelvic pain. This is particularly helpful in the patient who is afebrile at the onset of therapy. Another frequently employed measure is the disappearance of pelvic masses after therapy. All of these measures are helpful in the assessment of the patient's general well-being, but they do not give information on the most significant endpoint of therapy, the future fertility of these treated women. There is good evidence that this is adversely affected in many patients because of irreversible damage to the tubes. One study demonstrated an increasing incidence of infertility resulting from tubal damage after repeated episodes of salpingitis[7] (Table V). This information is needed in the assessment of treatment regimens for women with salpingitis.

There is good evidence that laparoscopy would be a helpful technique in the evaluation of the outcome of therapy in women with acute salpingitis. Two published reports delineate the usefulness of this diagnostic technique. The first was published in 1965.[8] A study of the treatment of salpingitis was assessed at that time. The hypothesis analyzed was the potential favorable impact of adrenal corticosteroids upon the treatment of patients with acute salpingitis. Since these agents have known antiinflammatory properties, it was postulated that their routine use would reduce the inflammatory response and result in a pelvis with less permanent tubal damage. A similar antibiotic regimen was used in all women, with half receiving concomitant doses of steroids. All of the commonly employed methods of assessment of the acute response to therapy indicated superiority of the steroid therapy. These women became afebrile more rapidly and had more rapid resolution of both pelvic pain and pelvic masses. Based upon this evaluation alone, the conclusion would have to be that steroids should be employed in such women. Follow-up laparoscopy provided a definitive answer to the question of treatment efficacy. This procedure showed no difference in the end results of therapy. The incidence of blocked tubes and pelvic adhesion formation was no different in the two populations. This follow-up diagnostic procedure exactly defined the impact of steroid therapy and dampened any clinical enthusiasm for the routine employment of these potentially toxic therapeutic agents. Recently, laparoscopy was employed to evaluate the long-term effectiveness of a variety of antibiotic regimens including doxycycline, ampicillin, penicillin and streptomycin, or penicillin and chloramphenicol.* The hypothesis was that one regimen might be more effective than the others in preventing permanent tubal damage. Laparoscopy done after therapy showed no difference in the long-term results of the various antibiotic regimens. A better outcome was associated with milder infections and patients aged less than 26 years. This study does not exhaust the possibilities of antibiotic combinations, but the lack of difference in clinical response with the varied antibacterial coverage of the regimens employed does not generate enthusiasm for a best antibiotic therapy for all patients with salpingitis. More laparoscopy data are needed in the future to specifically address this question.

Better microbiologic understanding of salpingitis

There is a great deal of confusion about the microbiologic etiology of acute salpingitis. The major problem is the inaccessibility of the internal organs, the fallopian tubes, for direct microbiologic sampling. A number of studies reveal the shortcomings. There is poor correlation between endocervical and tubal cultures.[9] This is unfortunate because endocervical cultures are obtained so easily. Attempts to better delineate the bacterial nature of acute salpingitis have employed peritoneal fluid obtained by needle culdocentesis. The assumption here is that the peritoneal fluid usually is sterile, and that the bacteria recovered in women with salpingitis reflect the bacterial nature of the endosalpingitis. Indeed, Monif and associates[10] have championed a clinical classification of pelvic infections of endometritis-salpingitis-peritonitis, based to a large extent upon culdocentesis findings. Laparoscopy has demonstrated a major gap in the accuracy of culdocentesis. This should not have been unexpected for older studies showed a wide variation between tubal fluid findings and evaluations of the endosalpinx.[11] Sweet and co-workers[12] have shown marked discrepancies between peritoneal fluid samples and direct cultures from the fimbriated ends of the fallopian tubes. Mårdh and colleagues[13] demonstrated recovery of chlamydia from biopsies of fimbria at laparoscopy of patients with nongonococcal salpingitis. Clearly, these will not be routine diagnostic procedures, but such laparoscopy studies will add to our microbiologic understanding of salpingitis. Better microbiologic information may guide us to more accurate antibiotic selection.

Conclusions

There is a role for laparoscopy in the evaluation of women with salpingitis. The extent to which this procedure should be employed will be dependent upon documentation of the risks and benefits in large prospective studies. I would anticipate more widespread use of this operative technique in women requiring hospitalization for salpingitis.

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*Weström, L.: Personal communication.

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Differences in some clinical and laboratory parameters in acute salpingitis related to culture and serologic findings

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Women with laparoscopically verified acute salpingitis (AS) were studied, and 151 were classified as having: chlamydia-associated AS (C-AS), gonococcal-associated AS (G-AS), and nonchlamydial, nongonococcal-associated AS (NCNG-AS). Patients with G-AS were more often febrile (rectal temperature >38° C) and more often had a moderately elevated erythrocyte sedimentation rate (ESR) (16 to 30 mm/hr) compared to other patients. Women with NCNG-AS were more likely to have a normal ESR and a mild inflammatory reaction laparoscopically. C-AS women were more likely to have had pelvic pain for more than 3 days before seeking treatment and to have an ESR of >30 mm/hr on admission. Predisposing factors to AS, such as insertion of intrauterine device, hysterosalpingography, and curettage within 4 weeks of admission, were more common in the C-AS group. The tubal inflammatory changes in the C-AS group were generally more severe than expected from the relatively benign clinical course. (AM. J. OBSTET. GYNECOL. 138:1017, 1980.)

ACUTE PELVIC INFLAMMATORY disease traditionally has been divided into gonococcal and nongonococcal PID, based on the results of endocervical cultures for *Neisseria gonorrhoeae.*

Gonococcal-associated salpingitis differs from nongonococcal disease with respect to abnormal vaginal discharge, elevated temperature, and duration of pelvic pain on admission.[1, 2] In several series of PID patients the isolation rates of gonococci from cervical specimens have varied considerably, viz., between 0% and 80%.[3, 4]

In our hospital catchment region cervical gonorrhea in women with acute salpingitis (AS) has declined from 50% in 1965 to 16.7% in 1979. During recent years, cultural and serologic studies have indicated that *Chlamydia trachomatis* is a major etiologic agent in acute salpingitis.[5-7] The aim of the present study was to evaluate whether the history, clinical picture, and/or laboratory tests such as the ESR in chlamydial salpingitis differed from those in gonococcal salpingitis and/or nongonococcal, nonchlamydial salpingitis.

Material and methods

Diagnostic procedures. Laparoscopy was performed within 24 hours of admission to the hospital. The criteria for selecting the patients for laparoscopy, the laparoscopic technique, and the criteria for the diagnosis of acute AS were those described earlier.[1] The inflammatory changes in the fallopian tubes were graded as mild, moderately severe, or severe, according to a description published elsewhere.[8]

The technique used for the diagnosis of *C. trachomatis* was that described by Mårdh and associates.[5] *C. trachomatis* was cultured on cyclohexamide-treated McCoy cells.[9] The method used for the isolation and identification of *N. gonorrhoeae* was that reported by Mårdh and colleagues.[10] For the demonstration of serum IgM and IgG antibodies to *C. trachomatis*, a modified microimmunofluorescence technique was employed, using pools of chlamydial antigen.[11]

Patient material. Two hundred and forty-five women with laparoscopically verified acute AS were examined; 94 of these patients were excluded from the

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This study was supported by grant 16X-4509 from the Swedish Medical Research Council.

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Table I. Age distributions of women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

	Salpingitis group							
	C (n = 68)		*G (n = 19)*		*NCNG (n = 60)*			
Age group (yr)	*No.*	*%*	*No.*	*%*	*No.*	*%*	*Groups compared*	*Difference (p)*
≤19	23	33.8	6	31.6	19	29.7	All	NS*
20-24	28	41.2	8	42.1	15	23.4	C + G/NCNG	<0.05
25-29	10	14.7	2	10.5	12	18.8	All	NS
≥30	7	10.3	3	15.8	18	28.1	C + G/NCNG	<0.02
Median age	23		21		24			

*Not significant.

Table II. Duration of pelvic pain before admission in women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

	Salpingitis group							
	C (n = 68)		*G (n = 19)*		*NCNG (n = 64)*			
Pelvic pain (days)	*No.*	*%*	*No.*	*%*	*No.*	*%*	*Groups compared*	*Difference (p)*
1-3	10	14.7	6	31.6	24	37.5	C/G + NCNG	<0.01
4-6	19	27.9	5	26.3	10	15.6	All	NS*
7-9	11	16.2	4	21.1	13	20.3	All	NS
≥10	28	41.2	4	21.1	17	26.6	All	NS

*Not significant.

study. The 151 AS patients studied were divided into three groups: (1) patients with evidence of chlamydial (but not gonococcal) infection (C-AS), (2) patients with evidence of gonococcal (but not chlamydial) infection (G-AS), and (3) patients in whom neither chlamydial nor gonococcal infection was diagnosed (NCNG-AS).

C-AS. The chlamydial group comprised 68 patients. *N. gonorrhoeae* was not isolated from the cervix, the urethra, the rectum, or the fallopian tubes in these patients. Furthermore, they presented one or more of the following criteria: (1) a fourfold or greater change in titer of IgM and/or IgG serum antibodies to *C. trachomatis*, (2) a titer of IgM antibodies >1:32 and/or IgG antibodies >1:512 to *C. trachomatis*, and (3) *C. trachomatis* isolated from the fallopian tubes.

G-AS. This group comprised 19 women. These women fulfilled all three of the following criteria: (1) *N. gonorrhoeae* isolated from the cervix, the urethra, and/or the fallopian tubes, (2) *C. trachomatis* not isolated from the cervix, the urethra, and/or the fallopian tubes, and (3) titers of IgM and IgG antibodies to *C. trachomatis* of <1:8 and <1:64, respectively.

NCNG-AS. This group consisted of 64 patients. They fulfilled the following criteria: (1) *C. trachomatis* and *N. gonorrhoeae* not isolated from the cervix, the urethra, and/or the fallopian tubes and (2) titers of IgM and IgG antibodies to *C. trachomatis* of <1:8 and <1:64, respectively.

Patients excluded. The following patients were excluded:

1. From the C-AS group: patients from whom *N. gonorrhoeae* was isolated from the cervix, the urethra, the rectum, or the fallopian tubes (n = 8)
2. From the G-AS group: women from whom *C. trachomatis* was isolated from the cervix, the urethra, or the fallopian tubes (n = 7); patients with IgM antibodies to *C. trachomatis* of 1:8 to 1:16 and such IgG antibodies at a titer of 1:64 to 1:256, respectively (n = 12)
3. From the NCNG-AS group: patients with IgM and/or IgG antibodies to *C. trachomatis* at a titer of 1:8 to 1:16 and/or 1:64 to 1:256, respectively (n = 54)
4. Women from whom *C. trachomatis* was isolated from the cervix or the urethra, with IgM and/or IgG antibodies to *C. trachomatis* at a titer of <1:8 and <1:64, respectively (n = 13)

Anamnestic, laboratory and clinical data. The following data were recorded: age, gravidity, earlier episodes of AS (laparoscopically confirmed) and lower genital tract infection (LGTI), contraceptive method used, duration of pelvic pain before seeking treatment, complaints of increased vaginal discharge, irregular bleeding, urgency and/or pain at micturition, febrile illness (rectal temperature >38° C) and erythrocyte sedimentation rate (ESR in millimeters per hour) on

Table III. ESR on admission in women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

	Salpingitis group							
	C (n = 68)		*G (n = 19)*		*NCNG (n = 64)*			
ESR (mm/hr)	*No.*	*%*	*No.*	*%*	*No.*	*%*	*Groups compared*	*Difference (p)*
≤15	6	8.8	2	10.5	34	53.1	C/NCNG G/NCNG	<0.001 <0.01
16-30	18	26.5	11	57.9	18	28.1	C/G NCNG/G	<0.05 <0.05
>30	44	64.7	6	31.6	12	18.8	C/G C/NCNG	<0.05 <0.001

Table IV. Laparoscopic appearance of the tubes of women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

	Salpingitis group							
	C (n = 68)		*G (n = 19)*		*NCNG (n = 64)*			
Inflammatory reactions of the tubes	*No.*	*%*	*No.*	*%*	*No.*	*%*	*Groups compared*	*Difference (p)*
Mild	15	22.1	5	26.3	34	53.1	NCNG/C NCNG/G	<0.001 <0.05
Moderately severe	30	44.1	7	36.8	17	26.6	All	NS
Severe	23	33.8	7	36.8	13	20.3	All	NS

Table V. Distribution of some parameters in women with chlamydial (C), gonococcal (G), and nonchlamydial, nongonococcal (NCNG) salpingitis

	Salpingitis group							
	C (n = 68)		*G (n = 19)*		*NCNG (n = 64)*			
	No.	*%*	*No.*	*%*	*No.*	*%*	*Groups compared*	*Difference (p)*
Never pregnant	34	50.9	9	47.4	27	42.2	All	NS*
Previous LGTI	18	26.5	5	26.3	25	39.1	All	NS
Previous salpingitis	14	20.6	1	5.3	9	14.1	All	NS
Increased vaginal discharge	51	75.0	14	73.7	37	57.8	C + G/NCNG	<0.05
Irregular bleeding	27	39.7	5	26.3	19	29.7	All	NS
Urgency, frequency, and/ or pain at micturition	11	16.2	3	15.8	12	18.6	All	NS
Contraceptive method used								
IUD	28	41.2	11	57.9	22	34.4	All	NS
OC†	16	23.5	3	15.8	11	17.2	All	NS

*Not significant.
†Oral contraceptive.

admission, and the degree of inflammatory changes in the fallopian tubes.

Predisposing factors for AS, such as insertion of an intrauterine device (IUD), curettage, or hysterosalpingography (HSG) within 4 weeks of admission, were recorded also.

Statistical methods. The Yates correction of the chi-square test and Fischer's exact test were used.

Results

Teenagers made up approximately one third of the patients in each of the three AS groups (Table I). In the group aged 20 to 24 years, significantly more women belonged to the C-AS and G-AS groups than to the NCNG-AS group. More women were >30 years of age in this latter group than in the two other groups.

The patients in the G-AS and NCNG-AS groups more often had a short (<3 days) period of pelvic pain before seeking treatment compared to those in the chlamydia group (Table II). A high ESR (>30 mm/hr) was noted significantly more often in the C-AS group than in the other two groups (Table III). A normal ESR (<15 mm/hr) was observed significantly more often in the women in the NCNG-AS than in the C-AS and

Table VI. Relation between the titer of serum IgG antibodies to *C. trachomatis* and isolation of the organism from the cervix in women with chlamydial associated salpingitis

	C. trachomatis		
Titer	*Isolated (n = 23)*	*Not isolated (n = 26)*	*Difference (p)*
≤64	12	5	<0.05
128-256	6	4	NS*
≥512	5	17	<0.01

*Not significant.

G-AS groups. Mild inflammatory changes in the fallopian tubes were significantly more common in the NCNG-AS group than in the C-AS and G-AS groups (Table IV). In the G-AS group, 14 of 19 patients (73.7%) had febrile illness (rectal temperature >38° C) on admission. The corresponding figures in the C-AS group were 15 of 68 (22.1%) and in the NCNG-AS group, 19 of 64 (29.7%). These differences were significant ($p < 0.001$ and $p < 0.01$, respectively).

No significant differences between the three groups studied were observed regarding previous AS and LGTI or the type of contraceptive used at the time of infection (Table V). An increased vaginal discharge was reported by significantly more patients in the C-AS and G-AS groups than in the NCNG-AS group.

Insertion of an IUD, curettage, or HSG within 4 weeks of admission was significantly more common in the C-AS group than in the other two groups ($p < 0.05$). Such a measure had been undertaken in 17.3% of the C-AS patients and in 6.3% of the NCNG-AS patients, whereas none of the G-AS women had undergone such measures.

In the C-AS group there was an inverse relationship between the serum IgG antibody titer to *C. trachomatis* on admission and the isolation of this organism from the cervix (Table VI). *C. trachomatis* was isolated significantly more often from those patients who, on admission, had an IgG antibody titer of <1:64 than in those with a titer of >1:512.

Comment

To establish the microbiologic etiology of acute salpingitis by using culture and/or serologic techniques is time-consuming and cannot be performed in all instances. Therefore the clinician's initial therapeutic decisions often must be based on "bedside" clinical findings. Because chlamydia salpingitis is so common[5,7] it seemed worthwhile to evaluate whether the clinical picture and/or laboratory results presented by women with C-AS differed from those of G-AS and NCNG-AS patients. In order to present, insofar as possible, patients with evidence of purely chlamydial, purely gonococcal, and neither chlamydial nor gonococcal etiology for their infections, 94 (38.4%) of the cases were excluded. In these 94 patients the etiologic findings were equivocal.

The 151 women studied were divided into 68 cases with evidence of chlamydial etiology, 19 with gonococcal etiology, and 64 with neither chlamydial nor gonococcal etiology. The women with G-AS had febrile illness more often than the patients in the other groups and the duration of pelvic pain before seeking treatment was shorter than that for the C-AS group. These findings accord with earlier findings on gonococcal vis-à-vis nongonococcal salpingitis.[1,2]

The patients in the NCNG-AS group differed in several respects from the women in the other two groups. Their median age was greater, one out of four was 30 years of age or older. They did not report increased vaginal discharge as often as the patients in the C-AS and G-AS groups. Half of these women had a mild inflammatory reaction of the tubes and half had a normal ESR on examination.

The women who belonged to the C-AS group were younger than the NCNG-AS patients, but were older than the G-AS women. C-AS patients generally had experienced a more benign clinical course compared to G-AS and NCNG-AS patients. Women with chlamydial AS had a febrile illness less often and generally sought treatment after a longer period of pelvic pain than the women in the other two groups. On the other hand, the ESR on admission was highest in the C-AS group and the inflammatory changes in the fallopian tubes, as seen at laparoscopy, generally were more severe than expected from the relatively benign clinical picture. In this context, the findings of Henry-Suchet and Loffredo[12] are of interest. They isolated *C. trachomatis* from the fallopian tubes of women who had no history of salpingitis, but presented with tubal occlusion and signs of a "chronic active" tubal infection. Apparently, some cases of C-AS follow such a benign clinical course that they are not seen by doctors at all or, if so, only exceptionally at the hospital emergency room. This is of importance because of the destructive late effects on tubal function exerted by the chlamydial infections.

The clinical and laboratory appearance of the 94 women excluded from the study varied. The eight patients who fulfilled the criteria laid down for the C-AS group, but in whom *N. gonorrhoeae* was isolated from the cervix, appeared to be an intermediate group between the C-AS and the G-AS groups. These eight women had a short duration of pelvic pain and high ESRs. The 54 women with IgM and/or IgG antibody titers to *C. trachomatis* between 1:8 and 1:16 and/or 1:64 and 1:256, respectively, appeared as a mixture of the C-AS and the NCNG-AS patients with regard to age, duration of pelvic pain, ESR on admission, and degree of inflammatory changes in the fallopian tubes as seen at laparoscopy.

Of the total 245 women, *N. gonorrhoeae* was isolated from the cervix in 46 (18.7%). *C. trachomatis* was isolated from the cervix in 67 (33.8%) of 198 representative specimens. Both *C. trachomatis* and *N. gonorrhoeae* were isolated from the cervix in 10 patients.

Predisposing factors such as IUD insertions and curettage were more common in patients with C-AS than in the other two groups. This indicates that an uncomplicated cervical chlamydial infection might be overlooked more easily than, for instance, gonococcal cervicitis. Because chlamydial cervicitis is generally more prevalent than gonococcal cervicitis[13] and also presents more uncharacteristic signs of infection,[14] great care in excluding a chlamydial infection must be taken by all doctors performing procedures that could cause ascending spread of an infection of the lower genital tract.

On the basis of the clinical findings in cases of suspected salpingitis, one cannot with any confidence predict the etiologic agent in the individual case. The clinical and laboratory findings could, however, to a certain extent, guide the clinician in his choice of the initial diagnostic and therapeutic considerations. In this study, it was found that acute salpingitis caused by *C. trachomatis* often runs a comparatively benign clinical course. It is, therefore, of importance for all doctors who encounter such patients to be aware of the possibility of tubal chlamydial infection, to institute adequate antibiotic treatment, and to perform an epidemiologic follow-up of the patient's sexual partner(s).

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Microbiology of specimens obtained by laparoscopy from controls and from patients with pelvic inflammatory disease or infertility with tubal obstruction: *Chlamydia trachomatis* and *Ureaplasma urealyticum*

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We cultured for *Chlamydia trachomatis, Ureaplasma urealyticum,* and *Mycoplasma hominis* and performed chlamydial serologic studies in 99 women undergoing laparoscopy. These women included patients with acute salpingitis, infertile women with and without mild pelvic inflammatory disease, and controls. *C. trachomatis* infection was significantly more common in patients than in controls. We also identified low-grade "silent" PID among women with infertility resulting from tubal obstruction and suggest this may be caused by chlamydiae. (AM. J. OBSTET. GYNECOL. 138:1022, 1980.)

AEROBIC AND ANAEROBIC culturing of specimens obtained at laparoscopy from previously untreated patients with pelvic inflammatory disease identifies responsible organisms in 70% of cases.[1-3] Other microorganisms such as *Chlamydia trachomatis, Ureaplasma urealyticum,* and *Mycoplasma hominis* may be responsible for disease in the remaining patients.[4-6] Tubal occlusion or pelvic adhesions causing sterility are frequent sequelae of PID. In this study, we sought to identify microbiologic agents from laparoscopy specimens collected in controls and patients with acute PID or infertility owing to tubal obstruction. Histologic examinations have identified chronic inflammatory changes in the latter group of patients.[7]

Methods

All 99 study patients underwent laparoscopic examinations: (1) Seventeen women had acute PID; 13 of

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Table I. *C. trachomatis* in patients with acute PID or infertility with tubal obstruction and in controls, Paris

Diagnostic tests	Acute PID	*Infertility with tubal obstruction*		Total patients	Controls
		With mild inflammation	*No inflammation*		
No.	17	17	29	63	36
Serologic test					
No. tested	16	16	24	56	28
No positive (%)	9 (56)*	11 (69)**	12 (50)*	32 (57)**	5 (18)
Culture from peritoneum/tube					
No. tested	17	17	29	63	36
No. positive (%)	4 (23.5)*	6 (35.3)***	6 (20.7)*	15 (25.4)**	0 (0)
Culture from lower genital tract					
No. tested	16	13	29	58	22
No. positive (%)	6 (37.5)	3 (23.1)	9 (31)	18 (31)	3 (13.6)

Chi-square test using Yates' correction; comparison with controls. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

these patients had received antibiotics prior to examination, but no tetracycline had been administered. (2) Forty-six women were infertile owing to tubal obstruction but had no clinical evidence of PID; these women were scheduled for tuboplasty for tubal obstruction or pelvic adhesions. Seventeen had laparoscopic changes of mild inflammation and 29 had no inflammation. (3) The 36 control women were scheduled for laparoscopy either to evaluate ovarian causes of infertility or for other reasons.

Specimens were collected on swabs from the peritoneum and fallopian tubes at the time of laparoscopy. If possible, biopsy specimens of the tubes and adhesions were collected at the same time. In addition, specimens were obtained from the lower genital tract (urethra, endocervix, and vagina) and blood specimens were obtained. Specimen collection, culture, and serologic and microscopic techniques were those described previously.[2, 8-11]

The prevalence of serologic and cultural evidence of infection for patients as a whole and for patient subgroups was compared with the prevalence among the controls using the chi-square test with Yates' correction for continuity.

Results

Chlamydia trachomatis. Specimens for detection of *C. trachomatis* infection were processed at Fournier Institute Laboratory. Serologic tests for chlamydiae were performed in 56 patients and 28 controls (Table I). Controls were significantly less likely to have serologic evidence of chlamydial infection compared to all patients, or to any of the subgroups. Cultures for *C. trachomatis* were obtained from the pelvis and fallopian tubes of all 99 patients and controls. Chlamydia was significantly more common in all patient groups when compared with the controls. In contrast to isolation from the upper genital tract, recovery of chlamydiae from the lower genital tract was not significantly different between patients and controls.

Mycoplasmas. Specimens for isolation of mycoplasmas were processed by laboratories at the Fournier Institute and Saint Louis Hospital. *M. hominis* was not identified in specimens obtained laparoscopically from controls or patients. *U. urealyticum* was obtained from the peritoneum or fallopian tube in 17% of patients and 5% of controls (Table II). Differences between patient groups and the control group were not statistically significant. *U. urealyticum* was identified in the lower genital tract in 24% of patients and 18% of controls.

Other bacteriologic cultures. *Neisseria gonorrhoeae* was identified in one of the four patients with acute PID who had not received antibiotic therapy. This specimen was obtained from the fallopian tube and also contained *C. trachomatis*. An enterococcus was obtained by laparoscopy from another patient with acute PID. One laparoscopic specimen from an infertile patient with mild inflammation contained *Bacteroides fragilis*. All other laparoscopic specimens from patients and controls were negative on standard aerobic and anaerobic culturing.

Discussion

Mårdh and associates[4, 5] demonstrated the role of *C. trachomatis* in acute salpingitis when they recovered this agent from the peritoneal fluid or the tube in cultures from six of 20 patients. Our recovery of *C. trachomatis* from four of the 17 patients with acute PID further demonstrates the etiologic role of this agent in acute PID.

Mårdh and Weström,[6] in a study of 50 women with acute salpingitis, identified *M. hominis* in laparoscopic specimens from four patients and *U. urealyticum* in two patients. Other workers have not identified these organisms frequently in cases of salpingitis.[1, 3]

Other authors[1, 3] have identified organisms by stan-

Table II. Cultures for *U. urealyticum* in patients with acute PID or infertility with tubal obstruction and in controls, Paris

Culture site	*Acute PID*	*Infertility with tubal obstruction*			*Controls*
		Mild inflammation	*No inflammation*	*Total*	
No.	17	17	29	63	36
Peritoneum/tube					
No. tested	17	17	29	63	36
No. positive (%)	4 (23.5)	4 (23.5)	3 (10.3)	11 (17.5)	2 (5.6)
Lower genital tract					
No tested	16	13	24	53	22
No. positive (%)	3 (18.7)	6 (46.2)	4 (16.7)	13 (24.5)	4 (18.2)

No significant differences.

dard bacteriologic techniques more frequently than we have. In previous work, we obtained a variety of aerobic and anaerobic organisms from 70% of women with acute PID when specimens were collected and processed by the same techniques.[2] We think the virtual absence of such organisms in this series is due to prior use of antibiotics by 13 of the 17 PID patients.

All our infertile patients were free of abnormalities on clinical evaluations prior to laparoscopy. All these women were afebrile and had normal erythrocyte sedimentation rates. Only one patient had a history of prior PID; this patient was one of the 17 found to have mild inflammation at laparoscopy. She had a positive culture for *B. fragilis*. In contrast to the normal clinical examinations, 17 of these patients had visible inflammation at laparoscopy, including red peritoneum, viscous adhesions, abundant liquid in the pouch of Douglas, and inflammatory cysts near the tubes and ovaries. Recovery of *C. trachomatis* and serologic evidence of chlamydial infection were most common in the infertile patients with mild inflammatory changes.

Even infertile patients with tubal obstruction but without laparoscopic evidence of inflammation had significantly more serologic and cultural evidence of chlamydial infection. In addition, many of these patients had inflammatory changes in materials collected by laparoscopy and examined microscopically.

Our study not only identifies *C. trachomatis* as an important cause of acute PID, but also strongly suggests that *C. trachomatis* causes silent PID, which results in infertility. Other workers[12, 13] also have reported evidence of chlamydial infection in infertile women. The role of *U. urealyticum* is not established in this study, but our findings suggest further evaluation is needed. Other authors[14-16] also have suggested that *U. urealyticum* is a cause of PID and infertility. Since both chlamydiae and mycoplasmas respond to specific antibiotics, the recognition of this problem could lead to important advances in infertility management.

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Clinical aspects of acute pelvic inflammatory disease: Cook County Hospital

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We reviewed Cook County Hospital records of 134 consecutive patients hospitalized for acute pelvic inflammatory disease unrelated to intrauterine devices, delivery, or pelvic surgery. Nearly half our patients had peritonitis or a pelvic mass and most were young or nulliparous. *Neisseria gonorrhoeae* was recovered from the endocervices of 41 (34%) of 120 patients and was more common among younger patients. Most patients responded to antibiotic therapy; consequently, we recommend antibiotic treatment for acute PID unless surgical emergencies cannot be excluded or patients do not improve with antibiotic therapy alone. (AM. J. OBSTET. GYNECOL. 138:1026, 1980.)

ACUTE PELVIC INFLAMMATORY disease is a diverse symptom complex, resulting in part from *Neisseria gonorrhoeae* infection of the endocervix and extension of this infection to the fallopian tubes. Acute salpingitis may progress to cause peritonitis and pelvic masses or abscess.[1]

We reviewed the records of women hospitalized for acute PID to elucidate clinical aspects of PID, to identify the prevalence of *N. gonorrhoeae,* and to evaluate nonsurgical management of such patients.

Methods

We reviewed the records of patients admitted for acute PID between November, 1978, and July, 1979, to the gynecology service, Cook County Hospital, Chicago. Patients who wore an intrauterine contraceptive device (IUD), had undergone an abortion or surgical procedure, or had been delivered of an infant within the past 6 months were excluded. The 134 remaining patients constitute our study group.

We reviewed the records of these patients and abstracted the following: age, parity, duration between onset of pain and last menstrual period (LMP), prior PID history, admission temperature and total white blood cell count (WBC), duration of hospitalization, and clinical course. Patients were separated into two clinical groups on the basis of recorded findings: *uncomplicated PID*—these 70 patients had pelvic pain, adnexal and cervical tenderness, fever, leukocytosis, and an elevated erythrocyte sedimentation rate; *complicated PID*—these 64 patients had the aforementioned findings plus rebound tenderness; 18 of these also had a palpable, inflammatory pelvic mass.

Standard hospital procedures were used to process culture specimens from the endocervix to identify *N. gonorrhoeae*. Other laboratory tests also were done in the routine hospital laboratories.

Results

These 134 patients with acute PID constitute 6.5% of all admissions to the Cook County Hospital gynecology service during the 9 study months. Patients were young, 51% were 20 years of age or younger, and only 28% were 26 years or older. In addition, nearly half of the patients were nulliparous (46%) and only 22% had more than one child. Forty-four patients (33%) had previously had episodes of PID.

On admission, oral temperatures exceeded 38° C in 75% of those patients with complicated PID, but in only 54% of those with uncomplicated disease. Sixty-two percent of patients with complicated PID had a WBC exceeding 13,000 mm^3, while only 33% of those with uncomplicated disease had such leukocytosis.

Although gonorrhea cultures were obtained in all patients, culture results were recorded on only 120 patients' charts (Table I). On examination of cultures *N. gonorrhoeae* was identified in 24 (41%) of the 58 women with complicated disease but in only 17 (27%) of those with uncomplicated disease. Gonorrhea was significantly more common among younger women (chi-square test, $p < 0.05$) for patients overall but not for the subgroups. Gonococcal prevalence was not related

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Table I. Endocervical gonorrhea in women with acute PID categorized by severity of PID, age, and parity, Cook County Hospital, 1978-79

Severity group/gonorrhea culture results	*Age (yr)*				*Parity*			
	15-20	*21-25*	*≥26*	*Total*	*0*	*1*	*≥2*	*Total*
Uncomplicated PID (n = 70)								
Gonococcus positive	11	4	2	17	5	7	5	17
Gonococcus negative	18	13	14	45	18	14	13	45
No results	3	3	2	8	5	2	1	8
Complicated PID (n = 64)								
Gonococcus positive	18	3	3	24	16	7	1	24
Gonococcus negative	16	7	11	34	16	10	8	34
No results	2	2	2	6	1	2	3	6
All PID (n = 134)								
Gonococcus positive	29*	7*	5*	41	21	14	6	41
Gonococcus negative	34*	20*	25*	79	34	24	21	79
No results	5	5	4	14	6	4	4	14
Total	68	32	34	134	61	42	31	134

*Chi-square test, $p < 0.05$.

to parity, fever, leukocytosis, or time lapse since LMP.

Twenty-four (37%) of the patients with complicated PID were hospitalized for more than a week, while only 12 (17%) of those with uncomplicated disease stayed in the hospital for more than a week. Eight of the 18 patients with a pelvic mass required surgery to exclude surgical emergencies. All other patients responded to antibiotic therapy alone; those with uncomplicated disease responded most promptly. Response to treatment was not related to recovery of the gonococcus from the endocervix.

Discussion

We believe *N. gonorrhoeae* usually initiates acute PID; however, once the disease process is established, *Enterobacteriaceae* and anaerobes of the normal vaginal flora[2] may contribute to the disease process. Cultures obtained by laparoscopy or culdocentesis from the tube or pelvic peritoneum in patients with PID or from tubo-ovarian abscesses removed at surgery contain a variety of organisms.[3–7] For the most part Lip and Burgoyne[7] identified gonorrhea in cul-de-sac cultures obtained within 2 days of the onset of symptoms, but only rarely if symptoms were present for more than a week. Thus, we believe that the long interval between infection with *N. gonorrhoeae* and initial examination of our patients accounts for the low recovery of the gonococcus in this study.

Marked differences were noted between patients with uncomplicated PID and those with complicated PID. Despite the use of two or three antibiotics for the latter, patients with complicated PID responded more slowly and required prolonged hospitalization.

Our patient population was young and nulliparous; therefore, we attempted nonsurgical treatment unless we could not exclude a surgical emergency. This effort to save reproductive organs, albeit damaged, seemed justified by our results. No patient suffered rupture of a tubo-ovarian mass during the course of hospital therapy. It should be emphasized that we used drugs effective for *Bacteroides fragilis* and *Enterobacteriaceae* on the basis of reports that these organisms are associated with complicated PID.[3, 5–7]

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Puerperal infectious morbidity: Relationship to route of delivery and to antepartum *Chlamydia trachomatis* infection

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We investigated the relationship of route of delivery and of antepartum *Chlamydia trachomatis* cervical infection to selected forms of puerperal infectious morbidity, including intrapartum fever, or early (<48 hours) postpartum fever not attributable to infections of sites outside the uterus and late (48 hours to 6 weeks) postpartum endometritis. Infectious morbidity occurred in 27 (44%) of 62 women who underwent cesarean section and in 33 (10%) of 329 who underwent vaginal delivery ($p < 0.001$). Postsection infectious morbidity was not correlated with *C. trachomatis* infection and was largely limited to early postpartum fever. Among women who underwent vaginal delivery, infectious morbidity occurred in 10 (34%) of 29 women with and in 23 (8%) of 300 without *C. trachomatis* infection ($p < 0.001$), and chlamydial infection was associated only with intrapartum fever and late postpartum endometritis. Separate matched case-control analyses confirmed that cesarean section was associated with an increased risk of early postpartum fever ($p = 4 \times 10^{-8}$); whereas among women who underwent vaginal delivery, antepartum *C trachomatis* infection was associated with an increased risk of development of intrapartum fever or late postpartum endometritis ($p = 0.002$). (AM. J. OBSTET. GYNECOL. 138:1028, 1980.)

PUERPERAL INFECTION has occurred in 5% to 7.2% of women in recent studies,[1, 2] and a variety of aerobic and anaerobic bacteria and genital mycoplasmas have been isolated from women with puerperal pelvic infections.[3, 4] One recognized factor associated with an increased risk of postpartum infection is cesarean section. Postpartum pelvic infections have been reported to occur in as many as 43% to 65% of patients undergoing abdominal delivery.[5, 6] Organisms commonly isolated from the endometrium and/or the blood of patients with puerperal infection subsequent to cesarean section include *Escherichia coli,* group B streptococci, enterococci, peptococci, peptostreptococci, and *Bacteroides* species.[7]

Chlamydia trachomatis is a well documented cause of pelvic infections among nonpregnant women[8] and also may cause puerperal pelvic infectious morbidity among pregnant women. Although the role of *C. trachomatis* in maternal puerperal pelvic infections has not been studied systematically, ophthalmologists who were studying neonatal eye infections in the 1930s noted that a puerperal pelvic infection frequently occurred in the mothers of babies who developed neonatal conjunctivitis.[9] In

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Supported in part by a Program Project Grant (AI-12192) and a Training Grant (AI-07044) from the National Institutes of Health, and by a CCIC Grant (SEA 80-06-72) from the Division of Hospitals and Clinic, Bureau of Medical Services, United States Public Health Service.

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Table I. Characteristics associated with antepartum *Chlamydia trachomatis* isolation

	C. trachomatis *isolated (N = 32)*	C. trachomatis *not isolated (N = 359)*	*P*
Mean age in yr (range)	19.8 (15-37)	24.5 (14-25)	<0.0001
Mean gravidity (range)	1.7 (1-5)	2.4 (1-9)	<0.01
Welfare recipients *(%)*	25 (79)	133 (37)	<0.0001
Caucasians *(%)*	20 (64)	287 (80)	<0.01
Unmarried *(%)*	19 (58)	108 (30)	<0.0005

1976, Rees and associates[10] reported that 16 of 24 mothers whose babies had neonatal chlamydial conjunctivitis developed pelvic infection.

In 1974, we initiated a series of studies at the University of Washington to assess the relationship of *C. trachomatis* infection to perinatal and infant morbidity and to maternal puerperal morbidity. The present report concerns our initial analysis of the relationship between antepartum *C. trachomatis* infections, route of delivery, and maternal puerperal infectious morbidity. We found that cesarean section was closely associated with early postpartum fever, usually attributed clinically to endometritis, myometritis, and/or peritonitis; whereas antepartum *C. trachomatis* infection was closely associated with intrapartum fever, usually attributed clinically to chorioamnionitis, and with late postpartum endometritis.

Material and methods

Study population. From July, 1977, through June, 1979, 486 women attending the University of Washington Hospital prenatal clinic were asked to undergo cervical culture for *C. trachomatis* and standardized interview. The two research assistants assigned to explain the study and perform the interview were able to see the majority of women attending the clinic, although the constraints of time precluded enrolling all patients. All *C. trachomatis* infections detected during pregnancy were asymptomatic and were not treated. The 391 patients who underwent delivery at University Hospital and were followed through to the sixth-week postpartum visit, or until earlier diagnosis and treatment of puerperal pelvic infection, form the basis for this report.

Chlamydia isolation. Cervical cultures were obtained between weeks 32 and 36 of gestation, and in some cases at the first prenatal visit. Isolates were obtained from endocervical swabs which were placed in transport medium and frozen until inoculated onto 5-iodo-2-deoxyuridine–treated McCoy cells, as previously described.[11]

Diagnosis of puerperal pelvic infections. The diagnosis of puerperal pelvic infections was made by members of the resident staff who were unaware of the chlamydia culture results. We divided puerperal pelvic infections into the following categories:

1. *Intrapartum fever:* temperature ≥38° C during labor, uterine tenderness, and no apparent extrauterine source of fever. These infections were clinically attributed to amnionitis.
2. *Early postpartum fever:* uterine tenderness associated with temperature ≥38.5° C within the first 24 hours post partum[12] and/or temperature ≥38° C on two consecutive occasions 6 hours apart between 24 and 48 hours post partum, and with no apparent extrauterine source of fever. These infections all were clinically attributed to endometritis or, after cesarean section, to endometritis, myometritis, and/or peritonitis.
3. *Late postpartum endometritis:* lower abdominal pain and uterine tenderness, with or without fever, occurring between 48 hours and 6 weeks post partum, with no apparent extrauterine source of infection. These infections all were clinically attributed to endometritis.

Not included in our definition of puerperal pelvic infections were symptoms of urinary tract infection and cultures of urine that contained $\geq 10^5$ organisms/ml and infections at other sites, such as lung, abdominal incision, or episiotomy.

Data collection and analysis. At the first prenatal visit, we prospectively interviewed each subject to obtain demographic data (age, race, marital status, and welfare status) and a reproductive history. Charts of each patient were reviewed retrospectively to obtain variables associated with labor and delivery, such as use of intrauterine fetal monitoring, duration of labor, interval from rupture of membranes until delivery, hematocrit in labor, and mode of delivery (vaginal or cesarean section); and to ascertain the presence of puerperal infectious morbidity. The relationship of all variables to the occurrence of infectious morbidity and *C. trachomatis* positivity was assessed to determine which variables were confounding. Individual matching of each case with two controls was employed as a technique to control for confounding variables. A com-

Table II. Puerperal infectious morbidity in patients with and without antepartum *Chlamydia trachomatis* infection

	C. trachomatis *isolated (N = 32)*		C. trachomatis *not isolated (N = 359)*		
Infectious morbidity	*No.*	*%*	*No.*	*%*	*P*
Intrapartum fever	3	9	5	1	<0.025
Early postpartum fever (<48 hr post partum)	1	3	26	7	0.6
Late postpartum endometritis (48 hr to 6 wk post partum)	7	22	18	5	<0.005
Total infectious morbidity	11	34	49	14	<0.01

Table III. Puerperal infectious morbidity in relation to route of delivery

	Cesarean section (N = 62)		*Vaginal delivery (N = 329)*		
	No.	*%*	*No.*	*%*	*P*
Intrapartum fever	3	4.8	5	1.5	0.3
Early postpartum fever	22	35.5	5	1.5	<0.0001
Late postpartum endometritis	2	3.2	23	7.0	0.5
Total infectious morbidity	27	43.5	33	10.0	<0.001

puter program was used to randomly access the data file of women who did not experience specific forms of infectious morbidity in an attempt to find two matched controls for each woman with that form of infectious morbidity. A matched analysis allowing for a variable number of controls matched to each case was used,[13] and an analog of McNemar's test with the exact permutation distribution was used to determine the confidence intervals for risk estimates. Groups were compared by Student's t test or chi-square analysis.

Results

Chlamydia trachomatis was recovered from 45 (9.3%) of 486 women who underwent antepartum testing, including 32 (8.2%) of the 391 patients who underwent delivery at University Hospital and who were followed completely through 6 weeks post partum, or until earlier onset of puerperal pelvic infection. The general characteristics of these 391 patients in relation to antepartum *C. trachomatis* infection are listed in Table I. Patients with *C. trachomatis* infection were significantly younger, had lower mean gravidity, were more often receiving welfare support, were more often non-Caucasian, and were more often unmarried than patients without *C. trachomatis* infection.

Increased risk of intrapartum fever and late postpartum endometritis among women with antepartum *C. trachomatis* infection. Puerperal pelvic infections occurred in 60 (15%) of the 391 women who were studied. As shown in Table II, intrapartum fever occurred in three (9%) of 32 patients with and in five (1%) of 359 patients without antepartum *C. trachomatis* infection ($p < 0.025$). Late postpartum endometritis occurred among seven (19%) of 32 patients with and in 18 (5%) of 359 without antepartum chlamydial infection ($p < 0.01$). Early postpartum fever was not associated with antepartum chlamydial infection. All but two of the infants born to mothers with antepartum *C. trachomatis* infections were followed closely after delivery. No significant relationship was shown between risk of *C. trachomatis* infections in the infant and puerperal pelvic infection in the mother, although the sample was too small to exclude such a relationship with much confidence.

Increased risk of early postpartum fever after cesarean section. As shown in Table III, infectious morbidity occurred among 27 (43.5%) of 62 patients who underwent cesarean section vs. 33 (10%) of 329 patients who underwent vaginal delivery ($p < 0.001$). Intrapartum fever and late postpartum endometritis were not related to cesarean section. However, early postpartum fever occurred in 22 (35.5%) of 62 women who underwent cesarean section vs. five (1.5%) of 329 who underwent vaginal delivery ($p < 0.0001$).

Relationship of antepartum *C. trachomatis* infections to puerperal infection by route of delivery. A cesarean section was performed on three (9%) of the 32 patients with and on 58 (16%) of the 359 patients without antepartum *C. trachomatis* infection ($p > 0.4$). The relationship between infectious morbidity and antepartum chlamydial isolation is analyzed for each mode of delivery in Table IV. Antepartum *C. trachomatis* infec-

Table IV. Puerperal infectious morbidity in relation to route of delivery and antepartum *C. trachomatis* (CT) infection

	Cesarean section (N = 62)		*Vaginal delivery (N = 329)*	
Infectious morbidity	*CT+/total*	*% CT+*	*CT+/total*	*% CT+*
Intrapartum fever	0/3	-	3/5	60
Early postpartum fever	1/22	5	0/5	-
Late postpartum endometritis	0/2	-	7/23	30
No infectious morbidity	2/35	6	19/296	6

tion was not shown to be associated with puerperal infectious morbidity among patients delivered by cesarean section. (The number with *C. trachomatis* infections was small, and many received antibiotics for early postpartum fever.)

Among women who underwent vaginal delivery, puerperal infectious morbidity occurred in 10 (34.5%) of 29 with vs. 23 (8%) of 300 without antepartum *C. trachomatis* infections ($p < 0.0005$). Infectious morbidity was limited to intrapartum fever and late postpartum endometritis among those with antepartum *C. trachomatis* infection who underwent vaginal delivery.

Matched analysis. Five variables were found to be associated with both antepartum *C. trachomatis* infection and puerperal infectious morbidity: age, race, parity, marital status, and welfare support. Variables eliminated from further consideration because they were not associated with either *C. trachomatis* infections or puerperal infectious morbidity included: use of an internal fetal monitor, duration of labor, admission hematocrit, and duration of ruptured membranes prior to delivery. An attempt was made to match each woman who had any of the three forms of puerperal infectious morbidity with two controls who did not have that form of infectious morbidity, by means of the following matching criteria: *age* in years: <20, 20 to 24, 25 to 29, ≥30; *race:* Caucasian or non-Caucasian; *parity:* previous pregnancy or no previous pregnancy; *marital status:* married or not married; *welfare support:* receiving welfare payments or not receiving welfare payments.

Relationship of cesarean section to early postpartum fever—matched analysis. Twenty-four patients with early postpartum fever were matched for age, race, marital status, welfare status, and parity, with 46 women who did not develop fever. Controls could not be matched to three patients, all of whom had undergone cesarean section. Cesarean section had been performed in 19 (79%) of the 24 women who developed early postpartum fever and in only four (9%) of 46 controls without fever. The estimated relative risk for the development of early postpartum fever among women who underwent cesarean section compared to women who underwent vaginal delivery was infinitely higher, since in no instance did a control subject undergo cesarean section when a matched patient did not ($p = 4 \times 10^{-8}$). The lower boundary of the 95% confidence interval for estimated relative risk was 5.4

Relationship of antepartum *C. trachomatis* infections to late postpartum endometritis—matched analysis. Twenty-eight patients with intrapartum fever or late postpartum endometritis who underwent vaginal delivery were matched for the five factors with 53 women who underwent vaginal delivery and did not develop such infectious morbidity. Matched controls were not available for one patient. *C. trachomatis* was isolated antepartum from 10 (36%) of the 28 women with intrapartum fever or late postpartum endometritis and from five (9%) of 53 matched controls. The estimated relative risk for the development of intrapartum fever or late postpartum endometritis was infinitely higher among women who underwent vaginal delivery and developed antepartum *C trachomatis* infection vs. those who did not develop antepartum *C. trachomatis* infection, since in no instance was *C. trachomatis* isolated from a control matched with a culture-negative case ($p = 0.002$). The lower limit of the 95% confidence interval for the estimated relative risk was 1.9.

Discussion

This study showed a significant correlation of cesarean section with early postpartum fever, and a correlation of antepartum *C. trachomatis* infection with intrapartum fever and with late postpartum endometritis among women who underwent vaginal delivery. Although others have not classified puerperal pelvic infections in exactly the same way, this classification appears to correspond to the patterns and etiology of puerperal pelvic infections that we have seen and affords plausible and testable hypotheses concerning the pathophysiologic features of these infections.

Intrapartum fever in this study was attributed clinically by the resident staff to amnionitis because of associated uterine tenderness and lack of evidence of extrauterine infection, although amniotic fluid was not subjected to examination for leukocytes or bacteria, and the histopathologic characteristics of the membranes were not studied carefully. The correlation of antepartum *C. trachomatis* infection with intrapartum fever suggests that this organism may produce amnionitis. Harrison and Riggin[14] have, in fact, shown that primary human amnion cells are highly susceptible to infection by *C. trachomatis* in vitro. Invasion of the chorioamniotic membranes may be one factor that contributes to preterm rupture of membranes and/or preterm onset of labor, and to perinatal mortality, all of which we have found to be correlated with antepartum *C. trachomatis* infections.[15] Of the three infants born to women with antepartum *C. trachomatis* infections who developed intrapartum fever, one died within 24 hours and one died 6 days after delivery, although none was delivered before 37 weeks' gestation.

The association of *C. trachomatis* infection with late postpartum endometritis but not with early postpartum fever is consistent with the possibility that chlamydial infection ascends from the cervix to the endometrium after delivery, in view of the organism's long replication time and apparently long incubation period in other genital infections, such as nongonococcal urethritis. Rees and associates[10] observed that the onset of pelvic pain varied between 13 and 38 days post partum in nine mothers of infants with neonatal chlamydial conjunctivitis, although other mothers were given antibiotics earlier for "infected lochia or pyrexia." It is noteworthy that 18 of 25 cases of late postpartum endometritis in our study were not associated with antepartum *C. trachomatis* infection, and the etiology of such cases remains obscure. These women were not examined by us, no standardized criteria for endometritis were used, and cultures for *C. trachomatis* or for other pathogens were not obtained at the time of diagnosis.

The strikingly close association of early postpartum fever with cesarean section leaves little doubt that cesarean section is responsible for this complication; the rapid development of fever after delivery is most consistent with peritoneal contamination by pathogenic bacteria present in amniotic fluid at the time of operation. In fact, the presence of organisms within the amniotic cavity at the time of operation is predictive of the development of subsequent infection only within 48 hours after delivery.*

Approximately 40% of the infants born to mothers with antepartum *C. trachomatis* infection develop inclusion conjunctivitis, and 10% to 20% develop chlamydial pneumonia.[16, 17] The present study suggests that about 30% of these mothers develop puerperal pelvic infections. Since the prevalence of maternal antepartum *C. trachomatis* infections at University Hospital is nearly 10%, we estimate that approximately 4% of infants born at University Hospital develop inclusion conjunctivitis, 1% to 2% develop chlamydial pneumonia, and 3% of the mothers develop puerperal pelvic infection associated with chlamydia infection. A relationship of antepartum *C. trachomatis* infection to prematurity and perinatal mortality also has been found,[15] as noted above. In view of the considerable maternal, fetal, and infant morbidity associated with maternal antepartum *C. trachomatis* infection, controlled studies of eradication of *C. trachomatis* infection during pregnancy are needed.

Finally, the microbial etiology and diagnostic features of intrapartum fever and of late postpartum endometritis, in particular, require further study. The present study used a retrospective chart review to reveal the clinical diagnosis made by staff members who were unaware of the chlamydia culture status of the patients. The etiology of intrapartum fever and late postpartum endometritis may be elucidated further by prospective systematic evaluation of the clinical and pathologic manifestations of amnionitis and late postpartum endometritis, in conjunction with comprehensive study of the amniotic and endometrial flora.

We appreciate the assistance of Wong Ho Chow with data analysis.

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*Eschenbach, D. A.: Unpublished data.

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Chlamydia trachomatis infection in Fitz-Hugh–Curtis syndrome

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We studied 23 patients with pelvic inflammatory disease associated with symptoms of pleuritic upper abdominal pain, characteristic of Fitz-Hugh–Curtis syndrome (FHC). A fourfold or greater change in antibody titer to *Chlamydia trachomatis* was demonstrated by microimmunofluorescence in 14; an IgG antibody titer ≥1:1,024 was seen in 13; and IgM antibody was demonstrated in 11. Twenty (87%) of the 23 FHC patients, including all of the 12 with paired sera obtained at least 6 weeks apart, had serologic evidence of acute *C. trachomatis* infection. *Neisseria gonorrhoeae* was isolated from seven (30%) of the 23 FHC cases, and *C. trachomatis* was isolated from three of 10. Two groups of matched controls were studied; one group with PID but without FHC, and the other without PID. A larger proportion of patients with FHC had serologic evidence of acute *C. trachomatis* infection than either of the two control groups ($p < 0.05$ for each comparison). Among those with antibody to *C. trachomatis*, the geometric mean antibody titer for the FHC group (1:724) was significantly higher than that for the PID group (1:138) or for the non-PID group (1:103). Thus, FHC is not solely attributable to infection with *N. gonorrhoeae*: most cases are associated with acute *C. trachomatis* infection. (AM. J. OBSTET. GYNECOL. 138:1034, 1980.)

AN INCREASINGLY broad spectrum of disease is being associated with *Chlamydia trachomatis* infection.[1, 2] In addition to its etiologic role in nongonococcal urethritis and cervicitis, *C. trachomatis* is now recognized as a common cause of pelvic inflammatory disease.[3] Perihepatitis, also known as Fitz-Hugh–Curtis syndrome (FHC),[4, 5] has been thought to be a complication of gonococcal PID.[4–6] Previous serologic findings from our laboratory showed evidence of acute *C. trachomatis* infection in nine of 11 Swiss cases of perihepatitis-peritonitis,[7] but no control groups were studied and isolation of *C. trachomatis* was not attempted. This article reports studies on 23 additional cases of FHC and on two matched control groups. One control group had PID but no FHC, and the other had no PID. The results further support an etiologic association of acute *C. trachomatis* infection with the Fitz-Hugh–Curtis syndrome.

Material and methods

Study population. Patients in Seattle who were suspected of having PID were referred by University of Washington physicians during the period 1972 to 1978. Each referred patient underwent a standardized interview and examination by one of us (D. A. E.)[8] The clinical diagnosis of PID was made in 442 patients with 523 episodes of PID, all characterized by pain in the lower abdomen of less than 3 weeks' duration, abdominal tenderness, and, on pelvic examination, moderate to severe tenderness maximally localized to one or both adnexal areas, with involuntary guarding proportional to the degree of tenderness. Seventeen FHC cases were identified among the 442 women referred for PID on the basis of pleuritic upper abdominal pain and upper abdominal tenderness. Six additional women were referred specifically because of suspected FHC, and all six had evidence of recent or current PID. Two separate groups of matched controls for the FHC cases

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This study was supported by Public Health Service research grants AI-16222 and AI-12192 from the National Institute of Allergy and Infectious Diseases and grant EY-00219 from the National Eye Institute.

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Table I. Summary of isolation and *C. trachomatis* micro-IF serologic results in patients with FHC (Seattle, 1972-79)

			Isolation		*IgM antibody*				*IgG antibody*			
							Fourfold				*Fourfold*	
Patient	*Age (yr)*	*Follow-up period*	*GC*	*CT (type)*	*Type*	*Highest titer*	*Rise*	*Fall*	*Type*	*Highest titer*	*Rise*	*Fall*
1	22	8½ mo	+	+(D)	D/J	512/256	X	X	Mul	8,192	X	X
2	16	3½ mo	–	–	D	32	X	X	D	32	X	
3	21	1½ mo	+	–	B	8		X	G	32		X
4	20	2 wk	+	–		0				0		
5	25	10 mo	–	–		0			D	128	X	X
6	22	6½ mo	+		D	32	X	X	D	4,096	X	X
7	21	Single	+			0			D	1,024		
8	29	3 wk	–		B	128	X		G	4,096	X	
9	25	1 yr	–			0			D	1,024	X	X
10	28	8 mo	–		D	32		X	B/ED	512	X	X
11	36	1 mo	+		D	32			D	256		
12	19	2 wk	–		D	64			B/ED	512		
13	20	3 mo	+			0			D	256		X
14	23	5 mo	–		D	64		X	D	1,024		X
15	21	2 wk	–	–		0			ED	256		
16	19	3 wk	–	–		0			B	4,096	X	
17	19	2 mo	–	–	D	512		X	B	2,048		X
18	22	6 mo	–	+	D	16		X	Mul	2,048		X
19	27	1½ mo	–	+(F)		0			F	2,048	X	
20	20	Single	–			0			ED	256		
21	19	Single	–			0			B	2,048		
22	21	Single	–			0			F	8,192		
23	21	Single	–			0			D	1,024		

Legend: GC = *Neisseria gonorrhoeae;* CT = *Chlamydia trachomatis;* Mul = multiple antibody types.

(FHC/PID group) were selected from among the other 425 PID patients without FHC (PID group), and from a group of women attending a venereal disease clinic who had no symptoms of abdominal pain and in whom the findings on bimanual examination were normal (non-PID group). Nineteen patients from the PID group and 18 from the non-PID group were matched with patients in the FHC/PID group by age, results of endocervical culture for *Neisseria gonorrhoeae,* number of sexual partners in the previous 6 months, and birth control method.

Isolation of *N. gonorrhoeae* and *C. trachomatis*. Evidence of gonococcal infection was sought by culturing specimens from the cervix and anal canal. Cervical swabs were directly inoculated on Thayer-Martin medium, and gonococci were identified by standard methods.[8]

For *C. trachomatis* isolation, x-irradiated or 5-iodo-2-deoxyuridine–treated McCoy cell cultures[9] were used for the earlier study specimens, and diethylaminoethyl-dextran–treated HeLa 229 cell cultures[10] for later specimens. The isolates obtained from cervical swab specimens were immunotyped by the microimmunofluorescence (micro-IF) method.[11]

Serologic tests. All sera obtained from the same patient were tested concurrently for *C. trachomatis* antibody by the micro-IF technique. Commercial fluorescein conjugates were used for indirect fluorescent antibody staining. Goat antisera to human IgM (μ-chain specific), to human IgG (γ-chain specific), and to human immunoglobulins (IgM, IgG, and IgA combined), all labeled with fluorescein isothiocyanate, were obtained in lyophilized form from Hyland Laboratories, Los Angeles, California. Each serum specimen was first screened for antibody with the use of a conjugate of antihuman IgM/IgG/IgA at three fourfold dilutions against eight antigens, including immunotypes CJ, A, H, I, K, B, ED, and GF.[12] Serum with positive antibody was then titrated for IgM and IgG antibodies (with separate conjugates of anti-IgM and anti-IgG) concurrently by testing serial twofold dilutions of serum against antigens selected from the results of the screening test. The antigens used were formalinized elementary bodies of *C. trachomatis.*[13] Thus, antibody titers to specific immunotypes in both IgM and IgG were sought to assess the kinetics and immunotype specificity of the antibody response in each patient.

Results

The FHC patients ranged in age from 16 to 36 years, but only four were over 25 years of age. Pleuritic pain was experienced either at the same time as (over half of

Table II. Summary of isolation and serologic findings in a patient with FHC

				Chlamydia trachomatis *micro-IF antibody*			
		Isolation		*IgM*		*IgG*	
Date	*Clinical findings*	*GC*	*CT(type)*	*Type*	*Titer*	*Type*	*Titer*
8/13/72	Onset abdominal pain						
8/17/72	PID	–	–	J	16	J	128
10/09/72	Asymptomatic, normal examination						
12/27/72	Recurrent abdominal pain						
12/29/72	PID	+		D	64	Mul	256
1/06/73	Onset pleuritic pain						
1/12/73	Laparoscopy—edema of liver capsule	–	+(D)	D	512	Mul	8,192
2/09/73	Normal	–		D	256	Mul	2,048
5/03/73	Normal	–		D	64	Mul	512

Legend: GC = *Neisseria gonorrhoeae;* CT = *Chlamydia trachomatis;* Mul = multiple antibody types.

Table III. Comparison of the results of *C. trachomatis* serologic evaluation in patients* with and without FHC

	FHC/PID	*PID*	*Non-PID*
No. of matched patients	19	19	18
N. gonorrhoeae isolated	7	7	6
C. trachomatis no. isolated/no. attempted (%)	3/10 (30%)	4/14 (29%)	0/6 (0)
Serodiagnosis of acute *C. trachomatis* infection based on any of the following criteria:	17 (89%)	9 (47%)	7 (39%)
Presence of IgM antibody	11	6	3
Titer change ≥fourfold (among paired sera available)	14 (18)	8 (19)	2 (3)
High titer, ≥1:1,024	10	4	3
Micro-IF antibody present	18	17	13
Geometric mean titer	1:724	1:138	1:103
Micro-IF antibody absent	1	2	5

*Patients are matched by age, isolation of *N. gonorrhoeae,* number of partners in previous 6 months, and birth control method.

the FHC cases) or subsequent to the onset of PID. The pleuritic pain had its onset after therapy had been initiated for PID in only two patients. Five FHC patients underwent laparoscopy. All five had laparoscopic evidence of salpingitis and signs of edema of the liver capsule; only one patient (case 5) had typical adhesions between the liver capsule and peritoneum.

Table I shows a summary of the isolation and serologic results obtained in the 23 FHC cases. *N. gonorrhoeae* was isolated from the cervix in seven (30%) of the 23 patients. *C. trachomatis* was isolated from the cervix in three (30%) of the 10 patients in whom cultures were performed.

A serodiagnosis of acute *C. trachomatis* infection was made in 20 of 23 (87%) cases on the basis of one or a combination of the following criteria: (1) the presence of IgM antibody[14, 15]; (2) change of fourfold or greater in titer of IgM and/or IgG antibody; and (3) IgG antibody titer ≥1: 1,024.[12]

Two or more sera obtained after onset of the disease were available from 18 patients, covering a follow-up period of 2 weeks to 1 year, and only a single serum specimen was available from the other five patients. A significant titer change (fourfold or greater) in IgM and/or IgG antibodies was found in 14 of 18 patients for whom paired sera were tested, including all of 12 in whom paired sera were obtained at least 6 weeks apart.

The peak antibody titers were >1: 1,024 in 13 patients.

Table I also shows that the rate of *C. trachomatis* infection as detected by culturing or serologic evaluation was the same in those with and without concurrent gonococcal infections, as shown by isolation of the organism.

IgM antibodies are more type-specific than IgG antibodies. Among 11 patients who had IgM antibody, seven were shown to have an immunotype specificity different from the corresponding predominant IgG antibody type. This suggests that in FHC patients there was a relatively high frequency of reinfection with organisms that had an immunotype specificity different from that of previous infections (heterologous reinfection).

An example of serologic evidence of heterologous reinfection is illustrated in the following case report, summarized in Table II.

Case report. G. H., a 22-year-old woman, was first seen with PID on August 17, 1972, 4 days after onset of lower abdominal pain. Cultures of endocervical specimens for *N. gonorrhoeae* and *C. trachomatis* were negative, but the serum contained IgG and IgM antibodies against *C. trachomatis,* type J. She was given 3.5 gm oral ampicillin plus 1.0 gm probenecid, followed by 500 mg ampicillin orally four times daily for 10 days; she showed rapid improvement and was normal on October 9, 1972. Symptoms of lower abdominal pain recurred on December 27, and on December 29, 1972, *N. gonorrhoeae* was isolated from the cervix and cul-de-sac. Again she was treated with oral ampicillin according to the same schedule, but 8 days later, on January 6, 1973, she developed pleuritic right upper quadrant pain. On January 12, *C. trachomatis,* type D, was isolated from the cervix; and in serial sera, a rise and fall occurred in IgM antibody against *C. trachomatis,* type D. Laparoscopic examination showed tubal erythema and edema, and edema of the liver capsule without adhesions. She subsequently was given 8 gm ampicillin per day intravenously for 3 days, followed by 2 gm ampicillin orally per day for 7 more days. She improved and was well on subsequent visits on February 9 and May 3, 1973.

Results in matched controls. The results of isolation and serologic evaluation in controls are shown in Table III. The FHC/PID and PID groups were each comprised of 19 patients, in seven of whom *N. gonorrhoeae* was isolated. *C. trachomatis* was isolated from 30% of FHC/PID patients and 29% of the PID patients. The non-PID group was comprised of 18 patients, six of whom had gonorrhea; of six non-PID patients in whom cultures for *C. trachomatis* were performed, none was positive. Because paired sera were available from 18 of the FHC/PID patients, and from all 19 of the PID patients, but from only three of the non-PID patients, data on changes in titers in paired sera could be evaluated for only the first two groups. A serodiagnosis of acute *C. trachomatis* infection, by any of the three criteria, could be made in 17 (89%) of 19 FHC/PID patients and nine (47%) of 19 PID patients ($p = 0.01$, by McNemar's test for matched pairs). Evidence of acute *C. trachomatis* infection, based on IgM antibody and/or an IgG antibody titer $\geq$ 1:1,024, was found in 15 of 19 FHC/PID patients vs. eight of 19 PID patients ($p = 0.066$, McNemar's test) and six of 18 non-PID patients ($p = 0.038$, McNemar's test). Among those who had antibody to *C. trachomatis,* the geometric mean IgG antibody titers measured within 2 months after diagnosis of PID, with or without FHC, were significantly higher for FHC/PID cases (1:724) than for PID cases (1:138) ($p < 0.05$, Student's t test). The geometric mean titers for FHC/PID cases also were significantly higher than for non-PID cases (1:103) ($p < 0.05$, Student's t test), but serial sera were not available for most of the latter group.

Discussion

Compared with Swiss patients reported by Muller-Schoop and associates,[7] in all of whom there was perihepatitis or peritonitis, as demonstrated by laparoscopy, the FHC cases in this study may be characterized as milder and/or diagnosed and treated earlier in their course, since only one of five studied by laparoscopy had the classical evidence of fibrous perihepatitis with adhesions between the liver capsule and the parietal peritoneum. Nevertheless, in both groups of patients we found a very high proportion with "acute" *C. trachomatis* antibody as defined by the presence of IgM antibody, fourfold titer changes, and unusually high antibody titers. The studies reported here with matched control groups add a dimension not available in the Swiss study, and make the evidence even more persuasive that *C. trachomatis* may cause the Fitz-Hugh–Curtis syndrome. Although cultures for *C. trachomatis* were not performed routinely, the three FHC patients from whom *C. trachomatis* was isolated represent the first culture-positive cases to be reported.

Even with currently improved cell culture techniques, the sensitivity of *C. trachomatis* isolation may be limited in women. We previously isolated *C. trachomatis* from only 11 of 26 PID patients who demonstrated a fourfold rise or fall in antibody titer to this agent,[8] and others[16] also have suggested that a single cervical culture may be insensitive in patients with PID who have serologic evidence of *C. trachomatis* infection.

Gonococcal infection has previously been implicated almost exclusively as the cause of FHC.[4-6] We have recovered gonococci from the cervices of 91 (45%) of 204 patients with acute PID,[8] but from only seven of 23 FHC/PID patients. The method used for isolation of *N. gonorrhoeae* in this study was estimated to be about 80% sensitive; only a small proportion of patients with culture-negative PID had serologic evidence of gonococcal infection or had sex partners with gonococcal urethritis.[8] The important conclusion from this study and our previous study of Swiss patients with perihepatitis-peritonitis is to seriously question the standard view that FHC is due exclusively to infection with *N. gonorrhoeae*, and to suggest that *C. trachomatis* is causally related.

It is of interest that neither *C. trachomatis* nor *N. gonorrhoeae* organisms have ever been recovered from the surface of the liver in cases of this intriguing syndrome. Thus the pathogenesis of FHC remains incompletely defined, but our serologic data have implications in regard to the pathogenesis of FHC. The fact that the immunotype specificity of IgM antibody differed from that of IgG antibody in seven of 11 FHC cases suggests that reinfection with a heterologous immunotype of *C. trachomatis* may particularly predispose to FHC. In an experimental model (*C. trachomatis* eye infection in the monkey), we showed that heterologous reinfection could lead to unusually severe disease.[17, 18]

The value of the micro-IF test for serodiagnosis of *C. trachomatis* infection in cases of FHC is well illustrated in this study. The importance of tests that are specific for IgM and IgG antibody for detecting acute infection is emphasized. We previously found that women attending a public venereal disease clinic had a high frequency of *C. trachomatis* antibody even in the absence of current symptoms.[1] Differentiation of "acute" antibody from antibody residual from previous infection is necessary in serologic diagnosis of *C. trachomatis* infection in association with current disease, especially in sexually active women.

Statistical analysis and matching were performed with the assistance of Jackie Benedetti, Ph.D., and Catherine Critchlow, M.S.

Addendum

After these data had been presented, a Swedish report was published which adds important further confirmation to the association of *C. trachomatis* with perihepatitis-peritonitis in women. Wølner-Hanssen and associates (Perihepatitis and chlamydial salpingitis, Lancet **1**:901, 1980) have reported four cases described as Fitz-Hugh–Curtis syndrome, from which they isolated *C. trachomatis* and demonstrated serologic response.

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Sacroiliitis in women—A late sequela to acute salpingitis

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Fifty-seven women who were treated for severe acute salpingitis between June, 1974, and June, 1976, were examined in 1978, 24 to 48 months after their first hospitalization. At the same time, 31 healthy women matched for age and parity were examined. All patients and control subjects were examined independently by a gynecologist and a rheumatologist. Forty-two of the patients and all control subjects were examined by quantitative ^{99m}Tc-pertechnetate scanning of the sacroiliac joints. These same joints also were examined by x-ray in the 57 patients. Sacroiliitis was documented in 39 of the 57 salpingitis patients (68%) and in one of the control subjects (3%), a difference which was statistically significant. There was no difference between the two groups in the presence of histocompatibility antigen HLA B27. The findings strongly suggest a correlation between severe acute salpingitis and sacroiliitis in women. (AM. J. OBSTET. GYNECOL. 138:1039, 1980.)

CHRONIC ARTHRITIS of the spine (ankylosing spondylitis and sacroiliitis) is much less common in women than in men; women usually constitute 4% to 10% of the clinical material.[1]

The diagnosis of sacroiliitis usually is based on radiologic examination of the sacroiliac joints. Radiographically visualized alterations in these joints often are difficult to interpret. Furthermore, these changes may not be apparent until 2 to 3 years after the onset of the disease; consequently, the method is of little value for diagnosing early disease. Finally, radiographs are unreliable in the grading of current disease activity.

In view of these shortcomings, a quantitative ^{99m}Tc-pertechnetate scanning (QTPS) method has been used to identify and grade inflammatory involvement of the sacroiliac joints.[2] This method allows detection of early sacroiliitis before radiographic evidence is definite. The technique also minimizes the radiation dosage, an important factor when the test is used to investigate women of fertile age.

Although some workers[3, 4] have found that sacroiliitis is related to urogenital disease in men, no such correlation has been made in women.[5] We examined the records of patients who were born between 1930 and 1960, and who were admitted to the Department of Rheumatology, Karolinska sjukhuset, Stockholm, Sweden, for seronegative arthritis. Forty-three percent of the patients with sacroiliitis were women. This high frequency of sacroiliitis in women and the increased frequency of acute salpingitis in Sweden[6] prompted this study, part of which was published previously.[7]

Material and methods

Ninety women who had been admitted to the Department of Obstetrics and Gynecology at the Karolinska Sjukhuset between June, 1974, and June, 1976, for acute salpingitis (World Health Organization 612.01-612.09) were identified from hospital records. Patients were included in the study if they fulfilled the following clinical criteria (n = 72): (1) admitted for their first episode of acute salpingitis, (2) fever >38° C on admission, (3) erythrocyte sedimentation rate (ESR) >20 mm, and (4) adnexal tenderness and palpable adnexal masses present at the time of the gynecologic examination. Other patients were included if a first episode of severe acute salpingitis was diagnosed at laparoscopy or laparotomy (n = 18).

In June, 1978, the 90 women were sent letters requesting that they take part in a follow-up study to evaluate possible late effects of their disease. Two women had moved abroad, and 10 had moved to other parts of Sweden and could not be included in the study. Ten women could not be found in the social register, three were pregnant at the time, and eight did not want to take part in the study. The remaining 57 women comprised the study group.

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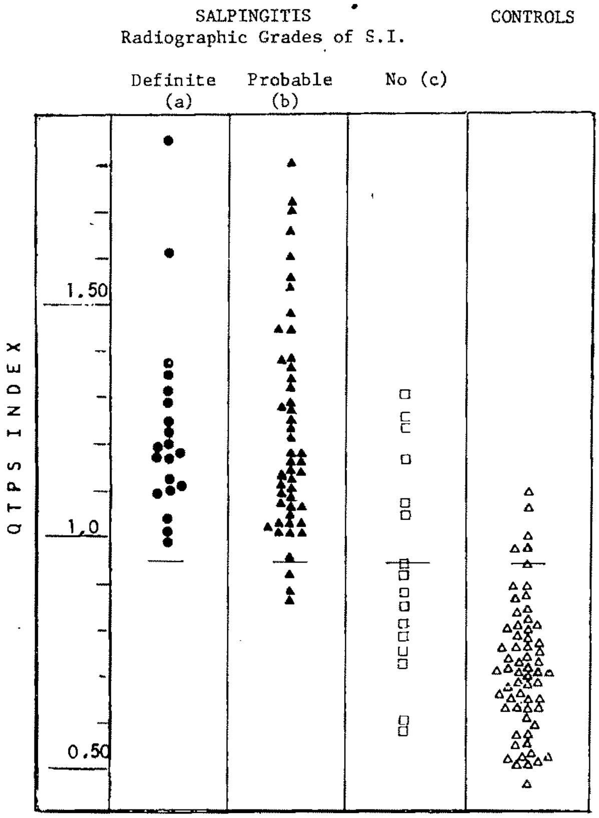

Fig. 1. Distribution of QTPS index for the control group and the salpingitis group, classified by the radiographic grade of sacroiliitis *(S.I.)*. QTPS was not performed in *(a)* five of the 15 patients with "definite" sacroiliitis, *(b)* two of the 26 patients with "probable" sacroiliitis, and *(c)* six of the 15 patients with no sacroiliitis. A *dash* indicates a normal QTPS index. (Reproduced with the permission of Almqvist & Wiksell, Int., Uppsala, Sweden, from Szanto and Hagenfeldt.[7])

Table I. Results of radiographic examination of the sacroiliac joints of 57 women 24 to 48 months after an episode of acute salpingitis

Sacroiliitis grade (New York criteria)	*No. of subjects*
Grade I-II (probable)	26
Grade III (definite)	15
Grade IV (complete fusion)	1
No changes	15

Treatment of the acute salpingitis had consisted of bed rest and antibiotic treatment with ampicillin and doxycycline for 10 to 30 days (mean, 18 days). *Neisseria gonorrhoeae* was the cause of the salpingitis in 15 of the 57 patients (26%). No culture of other microorganisms was performed at the time of the hospitalization.

Follow-up investigations done at 24 to 48 months after the first episode of acute salpingitis included the following procedures: (1) radiologic examination of the sacroiliac joints, with grading according to the New York criteria[8]: grade 0 = normal, I = suspicious changes, II = minimal abnormality of erosion and sclerosis, III = definite abnormality, and IV = complete fusion; we have combined I and II as "probable"; (2) QTPS of the sacroiliac joints[2]; (3) clinical examination by a rheumatologist; (4) clinical examination by a gynecologist; and (5) laboratory investigation, including ESR, hemoglobin, white blood cell count, microscopy of urine, tissue typing of histocompatibility antigens A, B, and C,[9] and culture of bacteria from the cervix.

A control group of 31 healthy subjects matched for age and parity who had not experienced pelvic inflammatory disease were selected at random from a group of women who were taking part in a clinical trial of a low-dose oral contraceptive pill. The control group underwent the same clinical and laboratory examinations as the study group, except that radiologic examination was not performed in the control group.

This study was approved by the Ethical Committee of the Karolinska Institute.

Results

The 57 study patients ranged in age from 19 to 34 years (mean, 27.5 years). Thirty-two women had never been pregnant, and 25 women had one to three children.

There were no differences in hereditary or predisposing factors between the two groups. However, 40% of the study group experienced lower-back pain and/or stiffness, whereas only 5% of the control group had such complaints.

In 15 patients, radiographic examinations of the sacroiliac joints were normal; 26 patients had "probable" sacroiliitis, 15 had "definite" sacroiliitis, and one had complete fusion of the joints (Table I). No radiographic examination was performed in the control women.

The QTPS was performed in 42 of the 57 study patients and in all 31 control women. Of the 42 patients investigated, 34 (81%) had an abnormally high QTPS index (Fig. 1). In contrast, only one of the 31 control women had an abnormal QTPS index. In four women with salpingitis but no radiologic abnormality, the QTPS method indicated inflammation in one or both joints.

Thirty (53%) of the 57 patients with salpingitis had been treated for recurrent salpingitis during the 24 to 48 months after the first episode. "Chronic salpingitis" was diagnosed by history and laparoscopy or laparotomy in eight women. At the follow-up gynecologic examination, all control subjects were healthy, except for one who had cervicitis. The culturing of specimens from the cervix identified the same frequencies of *N. gonorrhoeae* and *Chlamydia trachomatis* (10%) in both groups. A higher frequency of unspecified anaerobic microorganisms was identified in the salpingitis group. No other differences were found between the two groups with regard to ESR, white blood cell count, hemoglobin level, or urinary sediment.

Antibiotic therapy was given to all women for whom the cervical cultures were positive for *N. gonorrhoeae* or *C. trachomatis*. The women with proved sacroiliitis and clinical symptoms were given a nonsteroidal anti-inflammatory drug (Naproxen) for various lengths of time.

One year later, the symptoms in the 57 patients and in one of the control subjects who had sacroiliitis were reevaluated. Most women were symptom-free; however, seven women with definite sacroiliitis had incapacitating backache and stiffness. None of the women had developed spinal symptoms.

HLA B27 was found in 13 of the 57 women in the salpingitis group, and in seven of the 31 control women. HLA B17 and HLA B38 were found in three patients with sacroiliitis, one of whom also had psoriasis.

Discussion

At the first follow-up examination, 39 (68%) of the 57 women in the salpingitis group were considered to have sacroiliitis on the basis of the QTPS, the x-ray films of the sacroiliac joints, and the clinical symptoms. Only 18 of the 57 women had no manifest inflammation of these joints. In striking contrast, only one (3%) of the control women had an abnormal QTPS that indicated inflammation in the sacroiliac joints.

If one assumes that all 33 women (from among the original 90) who could not be examined were healthy, and if this assumption is correct, then the frequency of sacroiliitis 2 to 4 years after a first episode of severe acute salpingitis still would be as high as 43%. This figure is significantly higher than the 3% in the control group, indicating that sacroiliitis is a common late sequela of acute salpingitis.

The pathogenic role of various microorganisms in the development of complications of acute salpingitis is incompletely understood. In the present study, salpingitis associated with *N. gonorrhoeae* was no more likely to result in sacroiliitis than was nongonococcal salpingitis. Other chronic lesions of the fallopian tubes occur less frequently after gonococcal salpingitis than after nongonococcal disease.[6]

The frequency of HLA B27 in the salpingitis group was higher than in the normal population but lower than in men with uroarthritis.[10] This might indicate that the development of sacroiliitis in women who are suffering from salpingitis is not due to a genetic predisposition but may be caused by a spread per continuitatem, as suggested recently by Namey and Binndo.[11] Therefore, the common occurrence of sacroiliitis after acute salpingitis raises doubts about the adequacy of the antibiotic treatment at the time of the acute disease.

To further investigate the pathophysiologic features of sacroiliitis after PID, a prospective study is needed. This study should involve a meticulous microbiologic assessment at the time of the first episode of salpingitis, including all possible microorganisms from the fallopian tubes. Patients should receive adequate antibiotic treatment and be followed at various intervals by means of a low-irradiation technique, such as QTPS.

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The treatment of pelvic inflammatory disease

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The treatment of pelvic inflammatory disease depends upon the etiology of the condition. Pelvic infection (PI) after parturition and abortion, gynecologic surgery and a variety of invasive procedures is commonly associated with the isolation of anaerobic and aerobic flora of the vagina. The factors which influence the choice of antimicrobial treatment and the role of *Bacteroides fragilis* and *Escherichia coli* are discussed. Sexually transmissible agents of importance are *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Pelvic infections associated with these pathogens require antibiotics which exert an optimum effect against them. Examination and treatment of the sexual partner(s) are important. The possible role of the anaerobic and aerobic vaginal flora as opportunistic secondary pathogens is discussed. Developments in the surgical treatment of the sequelae of PID are reviewed. The results of treatment of uncomplicated gonorrhea in 262 women are reported. *C. trachomatis* was isolated from 53% of women before treatment. After treatment, PI developed in 11 women who had been given penicillin and in one woman who had been given tetracycline ($P = 0.0071$). It is suggested that recognition and treatment of postgonococcal cervicitis in women treated for uncomplicated gonorrhea with penicillin might provide one form of preventive treatment for nongonococcal PI. (AM. J. OBSTET. GYNECOL. 138:1042, 1980.)

THE LARGE AMOUNT of fundamental research into the etiology of pelvic inflammatory disease in recent years, particularly in Sweden and in the United States, has made us reexamine our basis for decisions regarding first-line antibiotic therapy for pelvic infection (PI). New surgical procedures have evolved, including microsurgical techniques, for the repair of damaged tubes and the electrocautery lysis of pelvic adhesions. This work is, in turn, being influenced by research in tubal physiology, particularly that relating to the role of the ciliated tubal epithelium and other factors affecting ovum transport.

This article briefly examines the medical and surgical aspects of treatment, as well as one facet of preventive treatment. Inflammation is not always due to infection. Trauma from surgical procedures can result in histamine release and the transudation of serum; organization of this response leads to the development of adhesions and scar tissue. Therefore, in speaking of medical treatment, the term PI rather than PID will be used.

Ideally, antibacterial treatment of any infection is based upon the identification of the causative organisms in specimens taken from the infected site(s) and the sensitivity of the isolates to antibacterial agents. In the case of PI, specimens from the fallopian tubes and pelvis can be collected only by laparoscopy. As a result specimens, particularly for sexually transmitted infections, taken from the lower genital tract or those obtained by culdocentesis are examined and are presumed to reflect the flora at the inflamed fallopian tubes.

Research during the past few years has led to the identification of *Chlamydia trachomatis* as an important sexually transmissible agent; also there is little doubt that it causes salpingitis. *Ureaplasma urealyticum* and *Mycoplasma hominis* also may cause salpingitis, although

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evidence is lacking. Important developments in anaerobic bacteriology have led to the recognition that many pyogenic infections are associated with a mixed flora which is predominantly anaerobic. Anaerobes have been recognized as important agents in infection associated with abortion and parturition, and also are important pathogens after gynecologic procedures or damage to the gut by operations or other trauma.

The great debate about PIs not attributable to abortion, parturition, or gynecologic or operative procedures has been stated elegantly by Cunningham,[1] on the one hand, and Eschenback and Holmes,[2] on the other. Do the aerobic and anaerobic bacteria isolated from peritoneal fluid obtained by culdocentesis ascend from their normal location in the vagina to the upper genital tract and initiate de novo an inflammatory process? If so, why? And why only in sexually active women? Or, on the other hand, are they opportunistic invaders secondary to inflammation caused by other primary pathogens from the cervix?

One could argue that, when the question of treatment is raised, it does not matter whether they are primary or secondary invaders. But it does matter. First, if they are secondary and other agents are assumed to be primary, then the treatment may not be appropriate for the causative organism. Second, failure to identify a sexually transmissible agent will lead to failure to treat the partner and, hence, to reinfection.

The need for directing treatment against all isolates in a mixed infection is not established. Certainly, in acute vaginitis due to *Trichomonas vaginalis,* a heavy growth of a large variety of aerobic and anaerobic organisms can be identified, yet treatment with metronidazole will restore the normal flora. Errors may arise through failure to identify a primary pathogen. For example, a report of "no bacterial growth" was made after routine microbiologic investigation of a swab collected from the eye of a newborn infant who developed purulent conjunctivitis. In similar cases the report may be "scanty normal flora" or "scanty *Staphylococcus albus*—?significance." Cell culture was necessary to identify the pathogen as *C. trachomatis.* Failure to eradicate one of two primary pathogens can result in the failure of initial treatment; recognition of this situation may be delayed by temporary suppression of one agent by the initial treatment. For example, an infant with conjunctivitis from whom both *Neisseria gonorrhoea* and *C. trachomatis* have been isolated will improve when treated with penicillin to eradicate the gonococcal infection. However, a low-grade conjunctivitis due to persistent *C. trachomatis* may continue and gradually recrudesce.

Similarly, a dual infection of the fallopian tubes might be diagnosed as gonococcal PI and treated appropriately for that particular pathogen. A residual chlamydial infection of the tube would remain, perhaps with secondary infection by anaerobic and aerobic vaginal and gut flora.

Table I. Causes of pelvic infection

- Sexually transmissible agents
  - *Neisseria gonorrhoeae*
  - *Chlamydia trachomatis*
  - ?*Mycoplasma hominis*
  - ?*Ureaplasma urealyticum*
  - ?Viruses
- Invasive procedures
  - Dilatation and curettage
  - Hysterosalpingography
  - Tubal insufflation
  - Termination of pregnancy
- Intrauterine contraceptive devices
- Abortion and parturition
- Pelvic peritonitis due to pathologic conditions in the gut
- *Mycobacterium tuberculosis*
- Tropical diseases

It is apparent that polymicrobial infections, particularly those involving the normal aerobic and anaerobic flora of the lower genital tract, are difficult to classify as pathogenic, opportunistic pathogenic, or commensal, and cause problems in treatment. There are many ways of classifying the etiology of PI. I have used the grouping in Table I because treatment and management are varied along these lines.

Medical treatment

PI associated with pregnancy and gynecologic and surgical procedures. Pelvic infections associated with pregnancy and gynecologic and surgical procedures are, in the majority of cases, caused by the normal anaerobic and aerobic flora of the vagina and follow a disturbance of the environment. The predominant anaerobes are members of the *Bacteroides* species, and *Bacteroides fragilis* has been isolated from up to 79% of the cases of genital tract infection in this group. The predominant aerobe is *Escherichia coli.* Work by Bartlett[3] with animal models suggests a distinctive role for these two organisms in infections that involve the peritoneal cavity. *E. coli* was associated with acute peritonitis, septicemia, and a high mortality rate, and *B. fragilis* with abscess formation.

Choice of treatment directed against anaerobes is influenced by the predominance of *B. fragilis,* since it is resistant to the penicillins, cephalosporins, aminoglycosides, and tetracyclines. Effective antibacterials include metronidazole, clindamycin, and chloramphenicol. Erythromycin has not been used widely, but there is at present little in vitro resistance to it. Older formulations of erythromycin had variable bioavailability;

new formulations may be more consistent. Significant side effects are associated with both clindamycin and chloramphenicol, although these are uncommon. Pseudomembranous colitis can follow the administration of clindamycin, and it should not be used when diarrhea is present. Resistance of some *Bacteroides* strains to clindamycin has been reported. Aplastic anemia subsequent to the use of chloramphenicol is usually fatal. Willis and Jones[4] have suggested that the drug of choice for *Bacteroides* is metronidazole because it is rapidly bactericidal for anaerobic organisms; achieves therapeutic levels in all body tissues and fluids after rectal, oral, and intravenous administration; has no detectable effect on gut flora; and is inexpensive, stable, and nontoxic.

Antibiotics which are effective against aerobes, including *E. coli,* are aminoglycosides, of which gentamicin and kanamycin are commonly used examples. A combination of metronidazole and gentamicin could be the first choice in PI after pregnancy or an invasive procedure. Alternatively, gentamicin with clindamycin or chloramphenicol may be used.

To avoid the need for a combination of antibacterial agents for mixed anaerobic/aerobic infections, Sweet and Ledger[5] evaluated the new cephamycin, cefoxitin. They reported that 92% of 109 patients responded to treatment, and that pelvic abscess which required drainage was the major cause of failure. However, Selkon[6] has expressed doubts about the efficiency of cefoxitin, on the basis of in vitro studies. He found that the relationship of pharmacokinetic parameters and the expected minimum inhibitory concentration was unsatisfactory. Furthermore, he observed that metronidazole produced sterile cultures within 6 hours and clindamycin within 30 hours, and that cefoxitin allowed regrowth to occur after an initial reduction. Clearly, further studies are necessary before substituting cefoxitin for combined treatment.

Even in the management of patients who appear to be in this group, consideration should be given to possible sexually transmissible agents. Paavonen and associates[7] have reported that among 51 patients who were admitted with PI associated with an intrauterine contraceptive device, *N. gonorrhoeae* was isolated from eight, *C. trachomatis* from nine, and both agents from two. Neither the combined treatment nor cefoxitin would eradicate the chlamydial pathogens.

Conclusion.

1. Drug(s) of choice: metronidazole and gentamicin
2. Alternatives: clindamycin or chloramphenicol and gentamicin
3. Dosages: metronidazole tablets, 400 mg three times daily; metronidazole suppositories, 1 gm every 8 hours; gentamicin (intramuscular), ≥60 kg—80 mg every 8 hours, or <60 kg—60 mg every 8 hours; chloramphenicol, 0.5 to 1 gm every 6 hours; clindamycin phosphate (intramuscular), 300 mg every 6 hours; clindamycin hydrochloride (oral), 300 mg every 6 hours

PI associated with sexually transmissible agents. *N. gonorrhoeae* and *C. trachomatis* are the most important sexually transmissible organisms recognized at present. The roles of *M. hominis* and *U. urealyticum* are not clear because of the high carriage rate in controls. There is some evidence, however, that *U. urealyticum* has a pathogenic role in a proportion of cases of nongonococcal urethritis (NGU) in men.

The isolation rate of *N. gonorrhoeae* from the cervix of women with PI varies from 10% to 20% in Sweden[8] and up to 85% in Memphis, Tennessee.[9] The isolation rate is related to the prevalence of gonorrhea in the community and the population studied. The proportion of nongonococcal PI to gonococcal PI varies accordingly.

Nongonococcal salpingitis is associated with sexual activity and multiple partners. For this reason, one might expect that sexually transmissible agents rather than normal aerobic/anaerobic flora were the primary pathogens. The latter may have an important role in secondary infection, particularly in abscess formation.

In the present state of our knowledge, I would suggest that treatment of nongonococcal PI not associated with pregnancy or invasive procedures should be with an antibiotic aimed at the agents of NGU, combined with an antianaerobic agent. Tetracycline with metronidazole is a suitable combination. The new formulation of erythromycin stearate, rosaramicin, may provide a single drug alternative because of its activity against anaerobes, including *B. fragilis,* but this treatment awaits evaluation. Treatment should be continued for 14 to 21 days, and the sexual partner should be examined and treated.

Benzyl penicillin (penicillin G), 1 megaunit every 6 hours, remains the treatment of choice in acute salpingitis associated with cervical gonorrhea in patients admitted to the hospital. Prophylactic metronidazole can be added in severely ill patients. Failure to respond significantly to treatment in 24 to 48 hours suggests the presence of β-lactamase–producing gonococci. Treatment failure or laboratory identification of β-lactamase–producing gonococci requires a change of treatment to a β-lactam antibiotic—cefuroxime, cefoxitin, and spectinomycin have all been successful. However, these are expensive and may not be available—tetracycline, erythromycin stearate, or co-trimoxazole are alternatives, although difficulties may arise with multiply resistant gonococci. The use of penicillin with the β-lactamase inhibitor clavulanic acid[10] may be possible in the future if a nontoxic formulation can be produced inexpensively. A preparation is now available for assessment. Follow-up rectal cultures are essential, since gonococci can persist in the rectum alone. Clinical assessment is important also. Minor symptoms and signs may indicate persistence of the agents of NGU or anaerobes if antibiotics that are ineffective or only partially effective against these organisms have been used. Appropriate treatment for 14 days is then advisable. Patients with subacute gonococcal salpingitis may be treated with an initial injection of benzyl penicillin, 1 megaunit, and oral ampicillin or amoxicillin, 500 mg four times daily for 7 days. Probenecid may be added when there is a high prevalence of chromosome-mediated penicillin resistance. Alternatively, tetracycline or erythromycin stearate, 500 mg every 6 hours for 7 days, can be used if multiply resistant strains of gonococci are not a problem. Metronidazole should be given to patients who present late with low-grade infection and swelling of the appendages. Rosaramicin is yet to be evaluated. Early results in the treatment of NGU were poor, but a new formulation has improved bioavailability.

Conclusion.

1. Drug(s) of choice
   A. *N. gonorrhoeae* isolated
      (1) Acute infection—sodium benzyl penicillin and metronidazole; alternatives—cefuroxime, cefoxitin, spectinomycin, and tetracycline hydrochloride, all with metronidazole
      (2) Subacute infection: amoxicillin, ampicillin, and probenecid; alternatives: oxytetracycline with metronidazole
   B. *N. gonorrhoeae* not isolated—oxytetracycline and metronidazole; alternatives—erythromycin stearate and metronidazole
2. Dosages: sodium benzyl penicillin, 1 megaunit every 6 hours; metronidazole suppositories, 1 gm every 8 hours; cefuroxime, 1 gm every 8 hours intramuscularly; cefoxitin, 1 gm every 8 hours intramuscularly; spectinomycin, 2 gm every 12 hours; oxytetracycline, 1 gm, then 500 gm every 6 hours; amoxicillin, 500 mg orally, then 250 mg every 8 hours; ampicillin, 1 gm orally, then 500 mg every 6 hours; probenecid, 1 gm every 6 hours; erythromycin stearate, 1 gm orally, then 500 mg every 6 hours

Surgical treatment

Colposcopy is used for diagnostic purposes, assessment prior to tuboplasty, the removal of ova for fertilization in vitro, and electrocautery of adhesions. Apart from emergency surgical procedures for ruptured tubal abscess and salpingectomy or drainage for pyosalpinx, the surgical treatment of PID attempts to restore tubal patency in those cases which are suitable for tuboplasty. Research in this field has resulted from the development of microsurgical techniques pioneered by Swolen,[11] in Sweden, and has been reinforced by the increasing number of women seeking advice about infertility. The procedures consist of salpingolysis (removal of tubal peritoneal adhesions which are causing partial obstruction); salpingostomy, which may be a simple fimbriolysis or the opening of a tube in the absence of fimbriae to aid in the pickup of ova; and excision of part of a tube. Ancillary measures, such as prosthetic devices, corticosteroids, and postoperative hydropertubation may be used. Such procedures are contraindicated in patients with tubes severely damaged following endosalpingitis. The procedures are less likely to be successful even in mild cases of this type than in cases of relatively normal tubal mucosa and extensive adhesions.

Cilia are thought to be a prerequisite for ovum transport in the ampulla. The role of cilia in rabbits has been demonstrated by Eddy and his colleagues.[12] Using microsurgical techniques, those investigators reversed 1 cm segments of rabbit oviduct and observed that the cilia in the reversed segment continued to beat in their original direction, whereas the cilia in the unreversed segment beat in the opposite direction. Ova transported to the junction were arrested and, if manually displaced into the reversed segment, were returned to the junction. However, Jean and his colleagues[13] have reported the occurrence of pregnancy in a woman with Kartagener's syndrome, in which there is no ciliary activity throughout the body. The absence of tubal cilia in this patient was confirmed subsequently when she presented for tubal ligation. This suggests that the role of cilia is not critical for ovum transport.

Comparisons between different techniques and different workers are virtually impossible owing to the variable case selection. Gomel,[14] using microsurgical techniques for tuboplasty, reported results similar to those of Swolen—a 90% postoperative patency rate in 41 patients followed for 1 year and a 29% intrauterine pregnancy rate.

Pauerstein[15] has stressed that patency is not necessarily equated with normal function. He and others have developed an ingenious method for measuring function in the rabbit oviduct, using radioactive microspheres which accurately mimicked normal ovum transport and an intrauterine detector of radioactivity. They are adapting the system for use in primates.

For the women with very severely damaged tubes,

Table II. Profile of women who developed posttreatment PI

Relevant factors	Treatment: Penicillin	Treatment: Tetracycline
No. of cases	11	1
Previous gonorrhea	2	0
Previous PID	1	0
Oral contraception	5	1
Intrauterine contraceptive device	2	0
Never pregnant	6	0
C. trachomatis isolated at initial treatment	8	1
C. trachomatis reisolated at follow-up visit	4	1
N. gonorrhoeae reisolated	0	0
Admitted	4	1
Minimal inhibitory concentration <0.125 U penicillin/ml	10	0

continuing developments in transplantation may provide a solution. If that fails, extracorporeal fertilization, which was achieved by Steptoe and Edwards,[16] in England, may be the only hope.

Conclusion. Laparoscopy and the development of microsurgical techniques have improved the fertility of women in whom the fallopian tubes have been damaged by PID.

Preventive treatment

Infection with multiple sexually transmissible agents is common. *C. trachomatis* has been isolated from 27% to 63% of women with cervical gonorrhea. In Liverpool, our isolation rate in such women is 53% (139 of 262 women). We have reisolated chlamydia from 84% of women infected with both agents who had been treated for uncomplicated gonorrhea with a single injection of penicillin and probenecid or ampicillin. Only 43% were chlamydia-positive in the first posttreatment test, 3 to 7 days after treatment, indicating temporary suppression by penicillin.

A clinical diagnosis of postgonococcal cervicitis (PGC), the equivalent of postgonococcal urethritis (PGU) in the man, was possible in only 30% of cases. We made the diagnosis of PGC when persisting endocervical mucopus was found and cultures for gonococci were persistently negative. PGU has been reported in up to 65% of men treated for gonorrhea with penicillin. In general, men receive treatment for symptomatic or asymptomatic PGU, whereas women are not treated for PGC because the condition is difficult to diagnose. These facts pose the following questions: (1) Is nongonococcal PI common after treatment for gonococcal PI because the agents of NGU remain? (2) Are recurrent attacks of nongonococcal PI common because penicillin and the cephalosporins are used frequently in treatment and both have poor activity against the agents of NGU? (3) In the event of antibiotics active against agents of NGU being administered, is the patient being reinfected by an untreated asymptomatic partner who has PGU after his treatment for gonococcal urethritis?

Using two treatment schedules, we compared the incidences of PI after treatment of 262 women for uncomplicated gonorrhea. Treatment A was one intramuscular injection of procaine penicillin, 1.2 megaunits, and either probenecid, 1 gm, or ampicillin, 1 gm, orally. Treatment B was the administration of oxytetracycline, 500 mg four times daily for 7 days. Patients were unselected, except that treatment B was given to patients who had a history of hypersensitivity to penicillin, and treatment A was given to patients who had difficulty in swallowing tablets. Clinical and microbiologic methods have been described previously.[17]

C. trachomatis was isolated from 52% of the 129 women who were given treatment A and from 54% of the 133 women who were given treatment B. The 129 women in group A ranged in age from 15 to 52 years; 70% were 24 years of age or younger. Eleven percent were married. Oral contraceptives were used by 58%, and an intrauterine contraceptive device by 5%; 59% had never been pregnant. The strains of *N. gonorrhoeae* isolated were relatively sensitive to penicillin (minimal inhibitory concentration <0.125 U penicillin/ml) in 73% of cases. There was a wide socioeconomic distribution: 27% were unemployed, 15% were unskilled workers, 23% were skilled workers, 4% were students, and 4% were professional women (nurses, teachers, social workers). Twenty-five percent were housewives. There was no significant difference between the two groups, with the exception that 59% of group A and 38% of group B had never been pregnant.

Routine follow-up of patients treated for gonorrhea consisted of three sets of tests carried out at 3 to 7, 14, and 21 days. Prolongation occurred when patients delayed reattendance. Chlamydia-positive patients were asked to attend additionally at 6, 9, and 12 weeks. Cervical swabs for culturing of *C. trachomatis* were taken on each follow-up visit from those women who were isolation-positive before treatment. In group A, 4% of women defaulted after treatment; 14% attended once, 21% twice, 38% three times, and 23% more than three times. The comparable figures for group B were 5%, 16%, 17%, 17%, and 45%, respectively. The period of observation in group A was less than 14 days in 24%, 15 to 28 days in 34%, and 29 to 90 days in 38%. Comparable figures in group B were 20%, 25%, and 50%, respectively. On reisolation, treatment for the chlamydial infection was withheld until the third negative posttreatment test for *N. gonorrhoeae* had been completed, then oxytetracycline, 250 mg four times daily for 7 days, was administered. This accounts for the shorter period of follow-up in women who received treatment A.

PI was diagnosed in 11 patients who had been given treatment A. The onset of pain was 11 to 35 days (mean, 15 days) posttreatment in the eight chlamydia-positive patients. The mean onset of pain was 6 days in the three chlamydia-negative patients. PI was diagnosed in only one patient (chlamydia-positive) who was given treatment B. This difference is significant ($\chi^2 = 7.243$, $P = 0.0071$).

N. gonorrhoeae was reisolated from none of the 12 patients who developed PI. *C. trachomatis* was reisolated from five patients before treatment with oxytetracycline, 500 mg four times daily for 14 days, was started because of a clinical diagnosis of PI. Details on these patients can be found in Table II.

These results suggest that *C. trachomatis* or other agents of NGU that are sensitive to tetracycline and that cause PGC also may cause posttreatment PI in some patients treated for gonorrhea with penicillin. The anaerobic/aerobic flora involved in nongonococcal PI is not, in general, sensitive to tetracycline. It is suggested that the use of tetracycline in the treatment of gonorrhea or immediately after treatment with penicillin or β-lactam antibiotics may provide one form of preventive treatment for nongonococcal PI.

Conclusion.

1. A course of oxytetracycline after routine treatment for uncomplicated gonorrhea may be administered to eliminate possible coexisting infection with the agents of nongonococcal urethritis
2. Alternative: erythromycin stearate
3. Dosage: oxytetracycline tablets, 500 mg every 6 hours; erythromycin stearate tablets, 500 mg every 6 hours.

Summary

In conclusion, I would say that the factors which need to be considered in the antibacterial treatment of PI must be assessed for individual patients and combined with information on antibiotic sensitivity of the organisms isolated or prevalent in the area and on the antibacterial agents available to the physician.

I have made no clear-cut statements about antibacterial therapy, and in this I have been influenced by the wisdom of that distinguished American physician William Osler, to whom is attributed the statement. "The greater the ignorance, the greater the dogmatism."

I am indebted to Dr. D. Hobson for the isolation of *C. trachomatis*. I am also indebted to Professor A. Percival for isolations and sensitivities of *N. gonorrhoeae*.

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Computer-assisted analysis of the therapy of acute salpingitis

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Isolation rates of microorganisms recovered by culdocentesis and/or laparoscopy in nine studies of salpingitis were multiplied by the proportions of each species which would be inhibited in vitro at peak and ½ peak serum levels of 13 antimicrobial drugs to yield a prediction of efficacy for the drugs. Efficacies of several hundred combinations of two and three of these drugs were estimated also. Some pairs of oral drugs were predicted to be as efficacious as some parenteral regimens of two or three drugs now in use. The predictions with this model parallel the results of some recently reported studies of therapy for salpingitis, and the model may prove to be a useful tool for future trials and therapy. (AM. J. OBSTET. GYNECOL. 138:1048, 1980.)

ACUTE SALPINGITIS is unusual among infectious diseases in its diverse microbiologic features. The difficulties of simultaneously considering the prevalences of the various organisms associated with the disease and their antimicrobial sensitivities led us to use a computer to weigh the theoretical merits of several hundred combinations of drugs.

Methods

We analyzed nine reports of microbiologic studies of acute salpingitis in which specimens for culturing were obtained either by culdocentesis or laparoscopy.[1–9] Although not strictly comparable in patient selection and diagnostic procedures, all the studies reported the results of microbiologic tests for a broad spectrum of organisms. Recovery of *Chlamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, Candida,* and viruses was not attempted in all studies. We calculated the prevalence of each organism by dividing the frequency of its isolation by the number of isolation attempts made in all the studies (ALL) (Table I) and in the studies using only laparoscopy (LAP)[6–8] (Table II). The average number of isolates per patient was 2.0 in ALL studies and 0.5 in LAP studies. Conspicuously absent from the laparoscopy isolates were *Bacteroides fragilis, Escherichia coli,* and β-hemolytic streptococci.

We selected 13 drugs that seemed to be useful in treating salpingitis, and from published sources derived anticipated peak serum levels (PSLs) for 23 variations in dose and route of administration[10–15] (Table III). We also used one half the PSL (½ PSL) as an estimate of tubal drug concentrations.[16] From yet other publications, we estimated the proportions of isolates inhibited in vitro at the dilution point of each drug, not exceeding the serum level under consideration.[9, 14, 17]* We selected approximately 450 combinations of two and three drugs which seemed to be practical and therapeutically compatible.[17] Drug synergy was considered only for the components of co-trimoxazole (considered as only one drug in this discussion); otherwise, the greatest probability of inhibition of the species by any drug in the combination was used. Microbial interactions were not considered.

An efficacy score for each regimen of one, two, or three drugs, expressed as percent of isolates inhibited, was derived by dividing the sum of the products of the individual organisms' prevalences and probabilities of inhibition (corrected with the angular transformation for proportions[18]) by the sum of prevalences:

$$\text{Efficacy} = \frac{\Sigma\,(\text{prevalence})\,(\text{probability of inhibition})}{\Sigma\ \text{prevalence}}\,.$$

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*Supported by *Maternal and Child Health Training Grant MCT00978 and **National Institutes of Health Research Grants AI-15642 and HD 13642.*

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Table I. Prevalence of isolates—ALL studies*

Organism	*Prevalence (%)*
Peptococcus sp.	27
Bacteroides sp.	22
Peptostreptococcus sp.	16
Viruses	13
Neisseria gonorrhoeae	13
Streptococcus, group D	9
Staphylococcus viridans	9
Escherichia coli	8
Mycoplasma hominis	8
Ureaplasma urealyticum	8
Streptococcus, ungrouped	7
Staphylococcus epidermidis	6
Corynebacterium sp.	6
Chlamydia trachomatis	6
Anaerobic cocci	5
Gaffkya sp.	4
Clostridium sp.	4
Streptococcus, group B	4
Propionibacterium sp.	4
Eubacterium sp.	2
Lactobacillus sp.	2
Corynebacterium vaginale	2
Veillonella sp.	2
Candida sp.	2
Fusobacterium sp.	2
Hemophilus influenzae	2
Staphylococcus aureus	1
Proteus mirabilis	1
Streptococcus, group A	1
Klebsiella sp.	1
Ruminococcus sp.	1
Proteus sp.	1
Acinetobacter sp.	1
Bifidobacterium sp.	1
Acidaminococcus sp.	1
Enterobacter sp.	<1
Aerobacter sp.	<1
Hemophilus sp.	<1

*Average, 2.0 isolates/patient.

Table II. Prevalence of isolates—LAP studies*

Organism	*Prevalence (%)*
Viruses	13
Chlamydia trachomatis	6
Mycoplasma hominis	6
Neisseria gonorrhoeae	5
Ureaplasma urealyticum	4
Hemophilus influenzae	4
Peptococcus sp.	4
Staphylococcus aureus	1
Staphylococcus viridans	1
Hemophilus sp.	1
Enterobacter sp.	1
Peptostreptococcus sp.	1

*Average, 0.5 isolates/patient.

Table III. Drugs analyzed

Drug	*Dose (mg)*	*Route*	*PSL ($\mu g/ml$)*
Ampicillin	500	Oral	3.8
	500	Intravenous	9.0
	1,000	Intravenous	40.0
Carbenicillin	2,000	Intravenous	125.0
	5,000	Intravenous	200.0
Cefoxitin	2,000	Intravenous	64.0
	2,000	Intravenous	32.0
Cephalexin	500	Oral	17.5
Cephalothin	2,000	Intravenous	100.0
Chloramphenicol	500	Oral	10.0
	500	Intravenous	10.0
Clindamycin	500	Oral	5.0
	300	Intravenous	15.0
Co-trimoxazole			
Trimethoprim	160	Oral	2.5
	6.7/kg	Intravenous	15.0
Sulfamethoxazole	800	Oral	50.0
	33.0/kg	Intravenous	225.0
Erythromycin	500	Oral	3.4
	500	Intravenous	10.7
Gentamicin	80	Intravenous	8.0
Metronidazole	500	Oral	10.0
	600	Intravenous	35.2
Penicillin G	3,000	Intravenous	16.8
Tetracycline	500	Oral	5.0
	500	Intravenous	15.0

Viruses were excluded from these and all subsequent calculations.

We calculated the variance for each efficacy score weighted for the prevalences of the bacterial isolates in the LAP studies. Using the 5% level of significance ($\alpha \leq 0.05$, two-tailed), we applied the Newman-Keuls test for comparison of multiple treatment means to the differences between efficacies.[19] We estimated sample sizes necessary for detecting differences with various degrees of certainty ($1-\beta$) with the method of Al-Bayyati.[20]

We compared the predictions of our model to the results of some recent trials of therapy for salpingitis.

Results

Predicted efficacies and sample sizes. Fig. 1 shows predicted efficacies for single drugs at ½ PSLs against LAP isolates. In this double matrix, the same drugs are listed in order of decreasing efficacy along both axes. The respective efficacies are shown only in the vertical axis, but are the same in the horizontal axis. Above and to the right of the diagonal, differences in efficacies between drugs along the axes are shown; sample sizes necessary for a 50% chance of detection of statistically significant differences ($1-\beta = 0.5$) are shown to the left and below the diagonal. Comparisons that are statistically different from each other are to the right of the vertical stepwise line (differences between efficacies) or below the horizontal stepwise line (corresponding sample sizes). To illustrate the difficulty of performing clinical trials of similarly efficacious regimens, we also

RANKED DIFFERENCE MATFIX (%)

REGIMENS RANKED (% EFFECTIVE)

%	TE	CB_5	C*	CB_2	CX	CT*	ER	CT	CL	TE*	AM	CC	ER*	PEN	AM	CC*	GM	AM*	CLX	MZ	MZ*
TE 84		0	1	2	4	5	5	5	6	10	10	12	14	15	15	16	16	17	33	70	71
CB_5 84	—		1	2	4	6	5	5	6	10	10	12	14	15	15	16	16	17	33	70	71
C* 83	1.9^4	1.9^4		1	3	4	4	4	5	9	9	11	13	14	14	15	15	16	32	69	70
CB_2 82	4.8^3	4.8^3	1.9^4		2	3	3	3	4	8	8	10	12	13	13	14	14	15	31	68	69
CX 80	1.2^3	1.2^3	2.1^3	4.8^3		1	1	1	2	6	6	8	10	11	11	12	12	13	29	66	67
CT* 79	766	766	1.2^3	2.1^3	1.9^4		0	0	1	5	5	7	9	10	10	11	11	12	28	65	66
ER 79	766	766	1.2^3	2.1^3	1.9^4	—		0	1	5	5	7	9	10	10	11	11	12	28	65	66
CT 79	531	531	766	1.2^3	1.9^4	—	—		1	5	5	7	9	10	10	11	11	12	28	65	66
CL 78	531	531	766	1.2^3	4.8^3	1.9^4	1.9^4	1.9^4		4	4	6	8	9	9	10	10	11	27	64	65
TE* 74	190	190	235	298	531	766	766	766	1.2^3		0	4	4	5	5	6	6	7	29	60	61
AM 74	190	190	235	298	531	766	766	766	1.2^3	—		2	4	5	5	6	6	7	23	60	61
CC 72	131	131	157	190	298	390	390	390	531	1.2^3	4.8^3		2	3	3	4	4	5	21	58	59
ER* 70	96	96	112	131	190	235	235	235	298	1.2^3	1.2^3	4.8^3		1	1	2	2	3	19	56	57
PEN 69	83	83	96	112	157	190	190	190	235	766	756	2.1^3	1.9^4		0	1	1	2	18	55	56
AM 69	83	83	96	112	157	190	190	190	235	766	766	2.1^3	1.9^4	—		1	1	2	18	55	56
CC* 68	73	73	83	96	131	157	157	157	190	531	531	1.2^3	4.8^3	1.9^4	1.9^4		0	1	17	54	55
GM 68	73	73	83	96	131	157	157	157	190	531	531	1.2^3	4.8^3	1.9^4	1.9^4	—		1	17	54	55
AM* 67	65	65	73	83	112	131	131	131	157	390	390	766	2.1^3	4.8^3	4.8^3	1.9^4	1.9^4		16	53	54
CLX 51	16	16	17	18	21	23	23	23	24	34	34	42	51	57	57	65	65	73		37	38
MZ 14	2	2	2	2	3	3	3	3	3	3	4	4	4	4	5	5	5	12	1.2		1
MZ* 13	2	2	2	2	3	3	3	3	3	3	4	4	4	4	4	4	5	11	11	1.9^4	

N_{50} SAMPLE SIZE MATRIX

*ORAL REGIMENS.

Fig. 1. Predicted efficacies for single drugs at ½ PSLs against organisms isolated by laparoscopy, with sample sizes necessary to show differences ($\alpha \leq 0.05$, $1-\beta = 0.5$). Regimens are ranked by their efficacies along the axes. The upper right half of the table shows differences between regimens. The lower left matrix shows the sample size necessary for a 50% probability of detecting a difference between regimens. Those differences to the right of the vertical stepwise line are significant by the Newman-Keuls test. Those values below the horizontal stepwise line are the sample sizes associated with the significant differences between regimens in the upper right half of the table. *TE,* tetracycline; *CB_5,* carbenicillin, 5 gm; *C,* chloramphenicol *CB_2,* carbenicillin, 2 gm; *CX,* cefoxitin; *CT,* co-trimoxazole; *ER,* erythromycin; *CL,* cephalothin; *AM,* ampicillin; *CC,* clindamycin; *PEN,* penicillin; *GM,* gentamicin; *CLX,* cephalexin; *MZ,* metronidazole. An *asterisk* indicates an oral regimen (chloramphenicol by either oral or intravenous route). Superscript numbers designate multiplication factor of 10 to power in superscript, e.g., $1.9^4 = 1.9 \times 10^4$.

have shown sample sizes for comparisons which we would not predict to be significantly different.

Great differences between the relative efficacies of drugs against ALL and against LAP isolates were sometimes noted. For example, intravenous tetracycline ranked first (tied with carbenicillin) against LAP isolates, but only fifteenth against ALL isolates. Serum levels in the ranges which we considered generally had little effect on efficacies against ALL or LAP isolates. Efficacies were identical for both doses of cefoxitin. The same PSL was used for either route of administration of chloramphenicol, the oral drug which was most efficacious against LAP isolates.

The 28 regimens most efficacious against LAP isolates are shown in Table IV. They were not significantly different from each other ($p > 0.05$). Of special interest was that two pairs of parenteral drugs—gentamicin with clindamycin or carbenicillin—and one combination of oral drugs—chloramphenicol, tetracycline, and co-trimoxazole—were as efficacious as the most efficacious combinations of three parenteral drugs. These highly efficacious regimens included parenteral drugs, co-trimoxazole, and metronidazole, which are not now commercially available in the United States.

Table V shows and compares the efficacies of several published regimens[21-32] and five combinations of oral drugs of our own invention (accompanied by § instead of a reference number) against LAP isolates. Each group of drugs bracketed on the right was significantly different in efficacy from the other bracketed group connected to it by a vertical line. The combination of oral co-trimoxazole and tetracycline (94%) was not significantly less efficacious than that of intravenous gentamicin and clindamycin with or without ampicillin (96%). This oral regimen was significantly more efficacious than the commonly used regimens of oral tetracycline, oral ampicillin, and parenteral ampicillin. Ampicillin, orally or at the lower intravenous dose (0.5 gm), also was significantly less efficacious than three other oral regimens: chloramphenicol with co-trimoxazole, chloramphenicol with tetracycline, and co-trimoxazole with clindamycin. Chloramphenicol with co-trimoxazole, not shown here, was the oral pair most efficacious against ALL isolates at ½ PSLs.

In Fig. 2, the same double matrix format used in Fig. 1, these same five oral pairs are compared with eight of the published regimens of Table V. The largest significant difference between efficacies, 29% (gentamicin with clindamycin with or without ampicillin vs. ampicillin orally), would require 21 patients in each treatment group for a 50% probability (1-β = 0.5) of demonstrating the difference. For a trial with a 90% probability of showing this difference (1-β = 0.9), 59 patients would be required in each treatment group. Only one microbiologic study of salpingitis had at least this many patients, i.e., 118.

Comparison with previous therapeutic trials. When applied to the isolates and prevalences in 30 hospitalized patients recently reported by Thompson and associates,[9] our model predicted efficacies of 81% for parenteral ampicillin and 92% for penicillin with gentamicin at ½ PSLs. Their observation that there was no difference in rates of improvement between treatment groups parallels our prediction that there is no statistically significant difference in efficacies between these regimens against their isolates.

If this difference of 11% between these regimens were really significantly different, we would estimate that each treatment group would require 157 patients in a study, with a 50% probability of demonstrating this difference. For a 90% probability of showing such a difference, 440 patients would be necessary in each group. Against our list of LAP isolates, 31 and 87 patients per treatment group would be required to show the difference of 24% between these regimens with 50% and 90% probabilities (1-β = 0.5 and 1-β = 0.9) respectively.

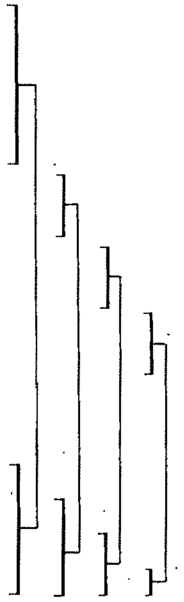
Table IV. Efficacies of 28 most efficacious regimens: assuming ½ PSLs and LAP isolates (all drugs given intravenously unless otherwise stated)

Regimen	*Efficacy (%)*
Carbenicillin 5 gm, erythromycin, clindamycin	97
Gentamicin, erythromycin, clindamycin	97
Tetracycline, co-trimoxazole, metronidazole	97
Gentamicin, co-trimoxazole, clindamycin	96
Gentamicin, co-trimoxazole orally, clindamycin	96
Erythromycin, co-trimoxazole, clindamycin	96
Erythromycin, co-trimoxazole orally, clindamycin	96
Chloramphenicol, tetracycline, co-trimoxazole	96
Chloramphenicol, tetracycline, co-trimoxazole orally	96
Chloramphenicol, tetracycline orally, co-trimoxazole	96
Chloramphenicol, tetracycline orally, co-trimoxazole orally	96
Chloramphenicol orally, tetracycline, co-trimoxazole	96
Chloramphenicol orally, tetracycline, co-trimoxazole orally	96
Chloramphenicol orally, tetracycline orally, co-trimoxazole	96
Chloramphenicol orally, tetracycline orally, co-trimoxazole orally	96
Gentamicin, clindamycin, ampicillin 1 gm	96
Gentamicin, clindamycin, ampicillin 0.5 gm	96
Gentamicin, clindamycin	96
Tetracycline, co-trimoxazole orally, metronidazole	96
Tetracycline, co-trimoxazole, metronidazole orally	96
Tetracycline, co-trimoxazole orally, metronidazole orally	96
Tetracycline orally, co-trimoxazole orally, metronidazole	96
Gentamicin, clindamycin, penicillin	96
Gentamicin, clindamycin, carbenicillin 5 gm	96
Gentamicin, clindamycin, cefoxitin 2 gm	96
Gentamicin, clindamycin, cefoxitin 1 gm	96
Gentamicin, carbenicillin 5 gm	96
Gentamicin, clindamycin, cephalothin	96

Cunningham and associates[33] compared results of treatment with oral tetracycline and oral ampicillin in 197 outpatients, in some of whom specimens for culturing were obtained by culdocentesis.[3] They did not attempt to isolate mycoplasmas or chlamydiae. Thirty percent of control culdocenteses yielded growth. They

Table V. Efficacies of published regimens and five oral pairs: assuming ½ PSLs and LAP isolates (all drugs given intravenously unless otherwise stated)

Regimen	*Efficacy (%)*
Ampicillin 0.5 gm, gentamicin, clindamycin[26]	96
Penicillin, gentamicin, clindamycin[27]	96
Clindamycin, gentamicin[29]	96
Co-trimoxazole orally, tetracycline orally*	94
Ampicillin 1 gm, gentamicin[25]	93
Penicillin, gentamicin[24]	93
Cephalothin, gentamicin[22]	93
Penicillin, clindamycin[26]	92
Ampicillin 0.5 gm, gentamicin[31]	92
Co-trimoxazole orally, chloramphenicol*	92
Chloramphenicol orally, tetracycline orally*	91
Co-trimoxazole orally, clindamycin orally*	90
Ampicillin 1 gm, clindamycin[26]	87
Ampicillin 0.5 gm, clindamycin[26]	86
Carbenicillin[30]	84
Tetracycline[31]	84
Tetracycline orally, metronidazole orally*	83
Cefoxitin[28]	80
Cephalothin[32]	78
Ampicillin 1 gm[23]	74
Tetracycline orally[21]	74
Ampicillin orally, clindamycin orally[26]	73
Penicillin[21]	69
Ampicillin 0.5 gm[21]	69
Ampicillin orally[21]	67



*Five hypothetical oral combinations.

found no significant difference between treatment groups in the duration of symptoms after treatment began.

Our estimates of the efficacies of ampicillin and tetracycline at ½ PSLs against their isolates are 85% and 41%, respectively, a difference which should be demonstrable with 90% ($1-\beta = 0.9$) probability using groups of 22 patients each. However, against our list of LAP isolates, these regimens were not significantly different ($p > 0.10$), and had they been, sample sizes of 390 each would have been required for a 50% chance of detection of the difference.

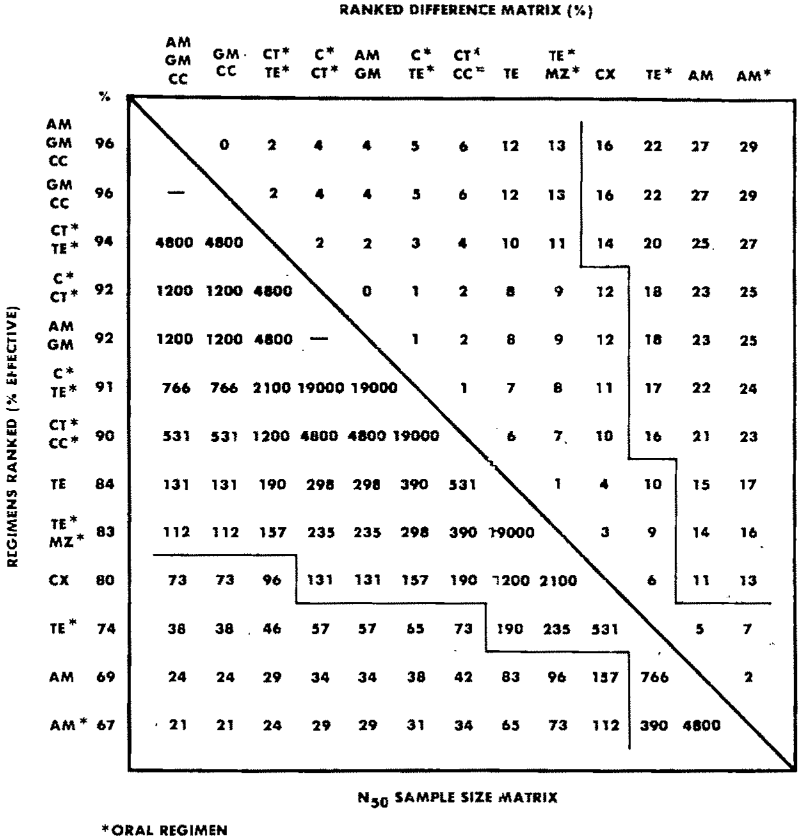
Discussion

Our model utilized published reports of in vitro effects of drugs tested by many methods and investigators, against microbial isolates from various diseases which occurred in geographically scattered patients over many years. Our assumptions about the interactions among drugs and microorganisms are necessarily simplistic because of the lack of information on the myriad possibilities in such a complex disease. Our method of predicting drug efficacy presupposes equal and independent pathogenicity of all microbial isolates, an assumption for or against which there is little experimental evidence.

The microbiologic features of salpingitis seem to vary greatly among the regions and populations in which salpingitis has been studied. We have emphasized the results of the culturing of specimens obtained by laparoscopy rather than culdocentesis while noting that the microbiologic results with the former technique have come from only two hospitals—in Lund, Sweden, and San Francisco, California.

In spite of these limitations, our model correlated well with the results of the study by Thompson and colleagues,[9] in which a broad range of microbial species was sought. Some of the discrepancy between our estimate of drug efficacies against our list of LAP isolates and against Thompson's isolates may have been due to the inherent sampling error in a study of only 30 patients, as well as to geographic, demographic, and temporal variations in the microbiologic characteristics of this disease.

Microbiologic technique partly explains the discrepancy between the observed relative efficacies of ampicillin and tetracycline in the study by Cunningham and associates[33] and our predictions of the efficacies of these drugs against their isolates.[3] If the cultures from patients with salpingitis were contaminated to the same extent as those of the controls, and if these contaminants were mostly vaginal anaerobes and coliforms, the predicted efficacy of ampicillin, which is more active against these organisms, would be exaggerated. If culturing for mycoplasmas and chlamydiae had been performed and had yielded organisms as frequently as in



RANKED DIFFERENCE MATRIX (%)

REGIMENS RANKED (% EFFECTIVE)	%	AM GM CC	GM CC	CT* TE*	C* CT*	AM GM	C* TE*	CT* CC*	TE	TE* MZ*	CX	TE*	AM	AM*
AM GM CC	96		0	2	4	4	5	6	12	13	16	22	27	29
GM CC	96	—		2	4	4	5	6	12	13	16	22	27	29
CT* TE*	94	4800	4800		2	2	3	4	10	11	14	20	25	27
C* CT*	92	1200	1200	4800		0	1	2	8	9	12	18	23	25
AM GM	92	1200	1200	4800	—		1	2	8	9	12	18	23	25
C* TE*	91	766	766	2100	19000	19000		1	7	8	11	17	22	24
CT* CC*	90	531	531	1200	4800	4800	19000		6	7	10	16	21	23
TE	84	131	131	190	298	298	390	531		1	4	10	15	17
TE* MZ*	83	112	112	157	235	235	298	390	19000		3	9	14	16
CX	80	73	73	96	131	131	157	190	1200	2100		6	11	13
TE*	74	38	38	46	57	57	65	73	190	235	531		5	7
AM	69	24	24	29	34	34	38	42	83	96	157	766		2
AM*	67	21	21	24	29	29	31	34	65	73	112	390	4800	

N_{50} SAMPLE SIZE MATRIX

*ORAL REGIMEN

Fig. 2. Predicted efficacies of 13 regimens at ½ PSLs against organisms isolated by laparoscopy: with sample sizes necessary to show differences between regimens ($\alpha \leq 0.05$, $1\text{-}\beta = 0.5$). Matrix format and symbols as in Fig. 1.

some other studies,[6-9] the predicted efficacy of tetracycline would have been increased. The extent of these distortions cannot be ascertained from the available data.

Without knowledge of the results of cultures obtained at laparoscopy, even a combination of seven drugs would not inhibit all strains of all possible isolates from the fallopian tubes. Evidence of the clinical usefulness of such cultures and susceptibility testing of isolates is lacking. The common practice (in the United States) of treating most patients with oral drugs as outpatients probably will continue. Their potential adverse effects aside, pairs of oral drugs should be more effective than ampicillin or tetracycline alone as treatment for these patients. Ideally, regimens from our model should be subjected to controlled clinical trials, but we envision possibly insurmountable problems in their implementation: elusive patients averse to invasive procedures, disagreeing diagnosticians, fastidious microorganisms, and variable delay between therapy and the ultimate test of cure—a normal pregnancy. Additionally, enormous resources and effort would be required for the recruitment of adequate numbers of patients, especially if they were matched for such variables as age, prior episodes of the disease, and microbiologic findings. Pending such studies, theoretically derived therapies may improve the prognosis of this complex disease.

We wish to thank Charles E. Irwin, Jr., M.D., George F. Brooks, M.D., and Richard L. Sweet, M.D., for material assistance and advice, and Alan G. Bostrom, Ph.D., for statistical consultation.

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Tubo-ovarian abscess: A retrospective review

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The charts of 160 patients with tubo-ovarian abscesses (TOAs) were reviewed. Patients were divided into two groups according to their response to initial medical therapy. Predictive factors were identified for the two groups. Prognosis was predictable on the basis of extent of disease at diagnosis and the initial response to medical therapy. There was no apparent association between a unilateral TOA and the use of an intrauterine contraceptive device. A minimum pregnancy rate of 8% was observed in patients maintaining reproductive function. No patient with a bilateral TOA conceived. Of all patients admitted to the hospital with TOA, 53% ultimately required surgical therapy. High residual morbidity and/or resultant infertility mandates more aggressive attempts at prevention. (AM. J. OBSTET. GYNECOL. 138:1055, 1980.)

THE INTRAABDOMINAL rupture of a tubo-ovarian abscess (TOA) has been one of the most serious complications of pelvic infections. Medical therapy of this condition has been associated with a mortality rate varying from 50% to 90%.[1, 2] In view of this, Vemeeren and TeLinde,[2] in 1954, and Mickal and Sellman,[3] as recently as 1969, have proposed that a total abdominal hysterectomy and bilateral salpingo-oophorectomy is the treatment of choice. This has been reported to result in a sevenfold decrease in deaths.[2]

The management of the patient with an unruptured TOA remains controversial and generally is decided on the basis of the patient's age, parity, and clinical circumstances. Although in the past TOA occurred primarily in an older age group, over the past several decades a progressively younger patient group has been encountered. The young patient with a TOA who desires to maintain reproductive function presents a significant dilemma to the gynecologist today.

We have conducted a retrospective study of patients with TOAs to define the epidemiologic and clinical factors associated with a response to medical management.

Material and methods

We reviewed the clinical histories of 160 patients admitted to the gynecology service of the Johns Hopkins Hospital with the diagnosis of tubo-ovarian abscess during the years 1969 to 1979. The diagnosis of TOA was based on the clinical presentation and confirmed by physical examination, sonography, diagnostic laparoscopy, or exploratory laparotomy. Patients in whom the admission diagnosis of a TOA was not confirmed, as well as those with postoperative pelvic abscess, were excluded from the study.

The management of patients with a TOA consisted of intensive intravenous antibiotic therapy and general supportive measures including fluids, nasogastric suction, and blood replacement when necessary. A response to medical therapy was defined as an absence of fever, a decrease in white blood cell count (WBC) by at least 5,000/mm^3, a decrease in the size of the mass, as well as improvement of symptoms. Surgery was reserved for cases of rupture, abscesses accessible to extraperitoneal drainage, and those cases refractory to medical management.

Patients were divided into two groups. Group I consisted of cases of TOA managed conservatively with intravenous antibiotics and demonstrating an adequate response. This group was then subdivided according to patients who did not require later surgery (group IA) and patients who underwent surgery during a read-

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Table I. Epidemiologic data

Group	*No.*	*Mean age (yr)*	*Nulliparity (%)*	*History of PID (%)*	*IUD (%)*
I (Medical therapy	110	25.5	26	53	51
IA (No subsequent surgery)	76	23	37	53	49
IB (Subsequent surgery	34	29	20	53	53
II (Immediate surgery)	50	30.8	32	42	40
Total of I and II	160	26.5	28	49	47

Table II. Clinical data on admission

Group	*No.*	*Fever > 100.4° F (%)*	*Size > 8 cm (%)*	*Unilateral (%)*	*WBC > 12,000 (%)*
I	110	76	46	66	75
IA	76	79	41	68	76
IB	34	71	56	62	73
II	50	74	80	34	73
Total of I and II	160	76	56	56	75

Table III. Clinical response to therapy

Group	*No.*	*Decrease in mass size (%)*	*Decrease in WBC (>3000) (%)*
IA	76	61	92
IB	34	41	62

Table IV. Surgical procedures

Group	*No.*	*TAH/BSO**	*Unilateral adnexectomy*	*Drainage*
IB	34	32	1	1
II	50	40	4	6

*Total abdominal hysterectomy/bilateral salpingo-oophorectomy.

mission for persistence or recurrence of disease (group IB). Group II included patients who required surgery during the acute phase because of failure to respond to the medical regimen.

Epidemiologic factors, clinical data, and hospital course were analyzed for each group and compared. An attempt was made to identify factors associated with failure of the medical regimen. Follow-up data were obtained on persistence and recurrence of pelvic infection. Patients not subjected to hysterectomy were analyzed for subsequent fertility.

The chi-square test was used for statistical analysis of the data.

Results

Group I consisted of 110 patients, 76 patients in group IA and 34 patients in group IB. Group II consisted of 50 patients.

Epidemiologic data (Table I). The mean age of patients responding to medical therapy (group I) was lower than that of those who did not respond (group II). Furthermore, a significantly larger number of nulliparous patients was noted among those responding to medical therapy. Incidence of a past history of pelvic inflammatory disease and use of an intrauterine contraceptive device (IUD) did not differ significantly between group I and group II patients.

Clinical data on admission (Table II). The incidences of fever (>100.4° F) and leukocytosis ($>12{,}000/mm^3$) were not different between the two groups of patients. On the other hand, a large TOA (>8 cm) was encountered more frequently in the surgically treated group ($p < 0.005$). A unilateral TOA was diagnosed more commonly in patients responding to medical therapy (group I) ($p < 0.025$). In addition, 51% of patients with a unilateral TOA used an IUD, whereas 61% of patients who used an IUD had a unilateral TOA.

Only four of 115 blood cultures were positive. All culture isolates were *Bacteroides* species. *N. gonorrhoeae* was cultured from the cervix of 25% and 19% of patients in groups I and II, respectively. Abscesses cultured at surgery were positive for at least one aerobic or anaerobic bacterium in 56% of cases.

Prognostic factors (Table III). Clinical response as defined by a decrease in temperature, WBC, and size of the mass was determined in each group. All patients in group I became afebrile. A decrease in the size of the mass was noted in 61% of patients in group IA and 41% of those in group IB ($p < 0.025$). A decrease in WBC occurred in 92% of individuals in group IA and 62% of those in group IB ($p < 0.005$). Patients in group II failed to defervesce and to meet one or more of these criteria. The mean interval from admission to

Table V. Follow-up

Group	*No.*	*Follow-up (%)*	*Mean duration of follow-up (mo)*	*Recurrence/persistence (%)*	*Pregnancies (%)*
I	110	86	26.5	86	9.5
IA	76	80	25.5	80	13.0
IB	34	100	28.8	100	2.9
II	50	76	25.9	12	0
Total of I and II	160	83	26.3	67	7.5

Table VI. Major surgical morbidity

Group	*No.*	*Wound infection*	*Cuff hematoma*	*Cuff abscess*	*Bowel injury*	*Pulmonary embolus*
IB	34	1	1	—	—	—
II	50	4	1	1	1	1

surgery was 5.5 days. Four ruptured TOAs were encountered, two of which were suspected at the time of admission.

Treatment (Table IV). Patients were treated medically with broad-spectrum antibiotics, with multiple agents being employed frequently. Surgery for refractory cases (group II) usually entailed total abdominal hysterectomy and bilateral salpingo-oophorectomy (80%). A unilateral adnexectomy was performed in four patients, while six patients underwent abscess drainage only.

Follow-up (Table V). Follow-up was possible for 83% of the patients. They were followed for 1 to 120 months. The average duration of follow-up was 25.5 months in group IA, 28.8 months in group IB, and 25.9 months in group II. Of the 61 patients in group IA who were followed, 80% had persistent or recurrent disease. Of these, 19 developed recurrent PID and four developed an acute TOA, while 26 had persistent pain, a residual mass, or adnexal thickening. Intrauterine pregnancies occurred in nine of 95 patients in group I who were followed. All occurred in patients with a unilateral TOA. There were no ectopic pregnancies.

There were two patients in group IB who were treated with conservative surgery (Table IV). One patient underwent unilateral adnexectomy without evidence of recurrent or persistent disease. The other patient was treated by colpotomy and responded, and diagnostic laparoscopy performed 4 years later for infertility revealed bilateral hydrosalpinx.

In group II, 10 patients underwent conservative surgery (Table IV). Of the four patients treated by unilateral adnexectomy, one patient required definitive surgery 3 years later, two patients did well, and one patient was lost to follow-up. Of the six patients treated by colpotomy, four had evidence of residual adnexal thickening, and two were lost to follow-up. There were two patients in whom an attempt was made to conserve one ovary. One had no recurrence in an 8 year follow-up, while 6 months later the other patient developed a recurrent abscess that required drainage. No pregnancies occurred in the 10 patients in group II who had undergone conservative surgery.

No patient in any group who underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy required additional pelvic surgery.

Surgical morbidity (Table VI). Abdominal wound infection was the most common surgical complication encountered (n = 5). A vaginal cuff hematoma was noted in two patients. A vaginal cuff abscess, large bowel injury, and pulmonary embolus occurred in one patient each. There were no deaths.

Group IB consisted of 34 patients requiring surgery for recurrent or persistent disease. Of these, 12 patients were readmitted with an acute TOA and failed to respond to medical therapy. The mean interval between initial medical therapy and surgery was 16 months.

Discussion

The definition of significant predictive parameters in patients with pelvic infections becomes more critical as increasingly younger patients are encountered. In the management of these patients, preservation of reproductive potential and ovarian function is an important consideration. Although there was a differential in age and parity between the medically and surgically treated groups, this might reflect a more refractory disease process in older patients as well as tendency toward more aggressive surgical therapy of the multiparous patient.

This study has clearly reemphasized the value of the extent of disease at initial diagnosis and the initial response to therapy in predicting outcome. Indeed, more advanced disease as defined by adnexal masses larger than 8 cm and/or bilateral adnexal involvement is predictive of failure to respond to medical therapy. However, the presence of fever and leukocytosis is of no predictive value. Furthermore, those patients requiring surgery subsequent to response to medical therapy (group IB) more frequently failed to show a decrease in mass size and WBC.

The incidence of IUD use was 47% for the total population. Interestingly, only 51% of the patients with unilateral disease and 41% of the patients with bilateral disease used IUDs. Both unilateral and bilateral disease can occur in the absence of an IUD, and IUD use is not associated with either unilateral or bilateral disease. This apparent lack of association between an unilateral TOA and use of an IUD has been reported previously.[4] In addition, the presence of an IUD did not have any prognostic significance. Similarly, a past history of PID was not predictive of response to medical therapy.

Classically, the management of patients with a ruptured TOA has been definitive surgery. However, more recently, less radical surgery has been suggested.[5] In this series, rupture occurred in four patients. The one patient treated conservatively did well, but of those patients with unruptured TOAs treated with conservative surgery, 21% required more definitive surgery.

The overall incidence of surgical complications was 12%, with no statistically significant difference between group IB and group II. However, more severe complications occurred in those patients undergoing operation in the acute phase of TOA.

Those patients treated by total abdominal hysterectomy and bilateral salpingo-oophorectomy had complete resolution of the disease process without necessity of further surgery. Of those patients who responded to medical therapy, 86% had persistence or recurrence associated with significant morbidity (groups IA and IB). Interestingly, of the 120 patients in whom reproductive function was preserved, nine conceived (7.5%). This is a conservative estimate as some patients were lost to follow-up, interval of follow-up varied, and some may have used contraception. In addition, pregnancies occurred in patients who had unilateral disease only.

Although a history of PID did not seem to have any prognostic value in assessing response to therapy, a TOA usually occurs in those with a background of PID. Clearly, patients with TOAs are seen more commonly in their childbearing years. The various modalities of treatment have always taken into account the patient's age and desire for future childbearing, but frequently have resulted in significant morbidity. The identification of prognostic factors, including the extent of disease and initial response to medical therapy, helps to differentiate between the surgically and non-surgically treated patients, but does not result in a reduction of morbidity. However, it should provide the basis for counseling and managing patients in these difficult and sometimes tragic situations. The exact role of surgical management, including the role of prophylactic adnexectomy in a young patient with unilateral TOA, needs to be determined on the basis of such factors. Although the value of a more aggressive form of management of young patients with unilateral TOA remains to be proved, it is clear that prevention of the development of such abscesses by more aggressive treatment of PID is crucial.

The authors would like to acknowledge John Reinhart, M.D., Ph.D., and Henry Sullivant, M.D., for help in abstracting the data for this article.

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Pelvic inflammatory disease and puerperal sepsis in Ethiopia

II. Treatment

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Where bacteriologic support is lacking, a combination of penicillin and streptomycin (P/S) is used empirically to treat serious gynecologic infections in Ethiopia. We compared the efficacy of P/S to a combination of penicillin and chloramphenicol (P/C) in hospitalized women who had bacteriologically confirmed pelvic inflammatory disease or puerperal sepsis (PPS). Treatment of PPS with P/C was significantly more effective in limiting the duration of fever than was treatment with P/S. Twenty-eight of 40 PPS patients had endometritis with retained products of conception that required evacuation of the uterus. The duration of fever in patients with PID who were treated with the P/C regimen was no different than that in those treated with P/S. Twenty of the 24 PID patients required surgery for drainage of abscesses, in addition to antibiotics. (AM. J. OBSTET. GYNECOL. 138:1059, 1980.)

PELVIC INFLAMMATORY DISEASE and puerperal sepsis (PPS) are the major causes of gynecologic morbidity at St. Paul's Hospital, Addis Ababa. In both infections the most commonly isolated organisms, in decreasing order of frequency, are *Enterobacteriaceae*, gonococci, *Streptococcus* species, and anaerobes. Most PID and PPS patients require surgery and a minimum of 10 days of antibiotic therapy in the hospital. A variety of antibiotics are used, but penicillin in combination with streptomycin (P/S) is the most popular. However, limited antibiotic sensitivity testing of bacteria isolated from PID and PPS patients indicates that penicillin or ampicillin in combination with chloramphenicol would be more effective than P/S.[1] This study compares the effectiveness of P/S and penicillin/chloramphenicol (P/C) for treating PID and PPS. We also sought to establish the indications for surgery and the type of operation most suitable for PID patients who were not cured by antibiotic treatment.

Materials and methods

Patient population. Twenty-four women with symptoms and signs of PID and 41 women with PPS attending the obstetric-gynecologic clinics at St. Paul's Hospital were referred to the Clinical Research Unit for study. The diagnosis of PID was made if the patient had fever, abdominal or pelvic pain, and a tender adnexal or pelvic mass. Peritonitis was found in nine patients. After obvious extragenital causes of infection were excluded, the diagnosis of PPS was made if the oral temperature was 38° C or greater for more than 48 hours after delivery. Two thirds of PPS patients had a

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The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of the Navy.

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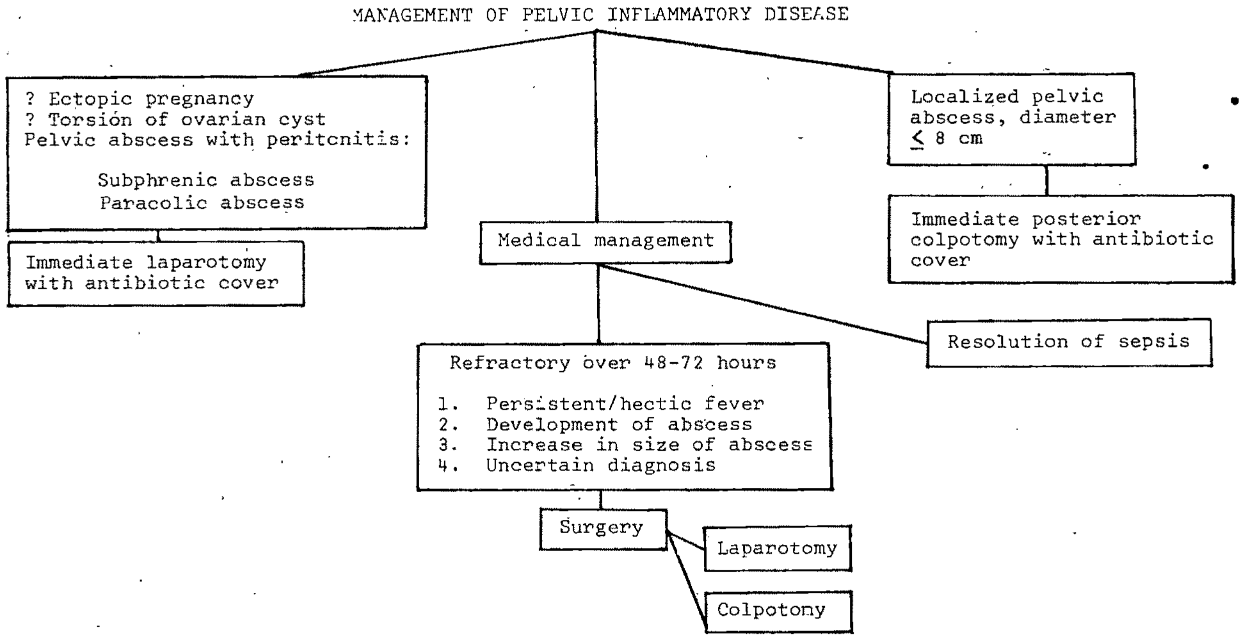
MANAGEMENT OF PELVIC INFLAMMATORY DISEASE
? Ectopic pregnancy
? Torsion of ovarian cyst
Pelvic abscess with peritonitis:
Subphrenic abscess
Paracolic abscess
Immediate laparotomy with antibiotic cover
Medical management
Localized pelvic abscess, diameter ≤ 8 cm
Immediate posterior colpotomy with antibiotic cover
Resolution of sepsis
Refractory over 48-72 hours
1. Persistent/hectic fever
2. Development of abscess
3. Increase in size of abscess
4. Uncertain diagnosis
Surgery
Laparotomy
Colpotomy

Fig. 1. Management of pelvic inflammatory disease.

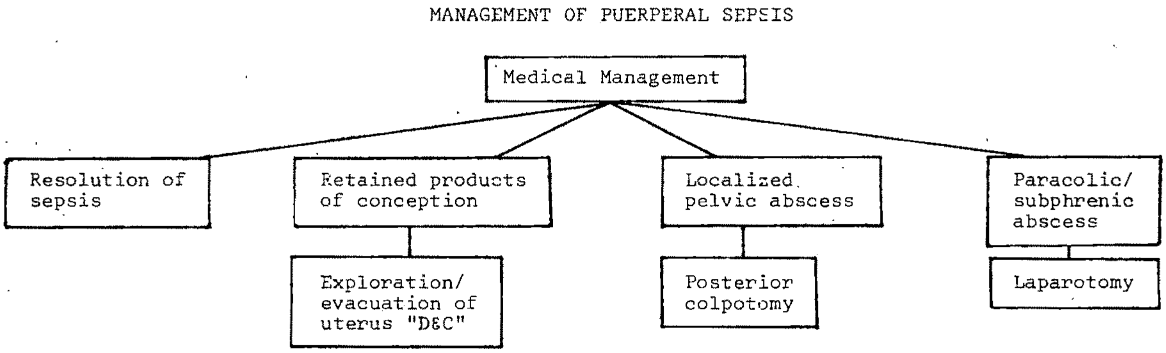
MANAGEMENT OF PUERPERAL SEPSIS
Medical Management
Resolution of sepsis
Retained products of conception
Exploration/ evacuation of uterus "D&C"
Localized pelvic abscess
Posterior colpotomy
Paracolic/ subphrenic abscess
Laparotomy

Fig. 2. Management of puerperal sepsis.

tender, subinvoluted uterus with excessive, offensive lochia. Peritonitis was present in nine patients. Each patient gave informed consent for treatment and, when indicated, for surgery.

Bacteriology techniques. The techniques used to collect, process, and cultivate specimens and to isolate and identify bacteria from PID and PPS patients have been described previously.[1] Bacteria isolated from blood, abscesses, and infected tissue were considered primary pathogens, and those isolated from the endocervix in patients with PID and PPS were identified as co-pathogens.

Treatment. No patient had received antibiotics in the week before admission. Patients were randomly allocated to receive treatment with either regimen P/C or P/S. Regimen P/C consisted of crystalline sodium penicillin G, 6 megaunits daily administered intravenously in six divided doses for 3 days, followed by aqueous procaine penicillin G, 1.2 megaunits daily administered intramuscularly for an additional 7 days, and chloramphenicol, 500 mg administered by mouth or intravenously every 6 hours for a total of 10 days. Regimen P/S contained the same penicillin dosage as the P/C regimen, but with intramuscular streptomycin, 1 gm daily for 10 days, substituted for chloramphenicol. Patients who showed no clinical improvement after 48 hours, or who had study drug–resistant bacteria by in vitro sensitivity tests, were prescribed alternative antibiotics. Gentamicin, 6 mg/kg intravenously, kanamycin, 7.5 mg/kg intramuscularly every 12 hours, or clindamycin, 30 mg/kg/day intravenously every 6 hours, was the usual alternative drug.

Surgery. Management of PID and PPS is outlined in Figs. 1 and 2. Laparotomy was performed in patients

Table I. Primary pathogen(s) isolated from patients with PID or PPS

Diagnosis	*Study group*				*Total*
	P/S		*P/C*		
	PID	*PPS*	*PID*	*PPS*	
No. patients	15	19	9	22	65
No. culture-negative	4 (26.7%)	6 (31.6%)	3 (33.3%)	5 (22.7%)	18 (27.7%)
Bacteria isolated					
N. gonorrhoeae	1	6(4*)	2 (1)	6 (1)	15
Enterobacteriaceae					
Escherichia coli	3 (1)	6 (4)	0	5 (4)	14
Proteus sp.	1 (1)	1 (1)	1 (1)	1 (1)	4
Klebsiella sp.	0	0	0	1 (1)	1
Salmonella typhi	2	0	0	0	2
Streptococci					
S. viridans	1 (1)	2 (1)	3 (3)	0	6
β-Hemolytic, group A	0	3 (1)	0	3 (2)	6
β-Hemolytic, other	2	0	0	1	3
S. pneumoniae	0	0	0	2	2
Unclassified	0	0	1 (1)	0	1
Anaerobes					
Bacteroides sp.	1	0	0	2 (1)	3
Clostridia sp.	1 (1)	0	0	0	1
Peptostreptococcus sp.	0	0	1 (1)	0	1
Miscellaneous					
Moraxella sp.	2 (1)	2 (2)	0	0	4
Staph. aureus	2 (1)	0	0	0	2
Pseudomonas sp.	0	0	0	1	1
Bacillus sp.	0	0	0	1	1
Haemophilus influenzae	1	0	0	0	1

*Number in parentheses indicates times isolated as a co-pathogen.

with free intraperitoneal fluid or with paracolic or subphrenic abscesses, if the diagnosis was in doubt (e.g., ectopic pregnancy or twisted ovarian cyst), and in patients with recurrent PID if bilateral salpingectomy was envisaged. Posterior colpotomy was done for localized pelvic abscess presenting as a well defined, tender, cystic or fluctuant mass in the cul-de-sac. Posterior colpotomy was preferred to laparotomy for infertile patients for whom elective surgery for tubal patency could be deferred. Puerperal patients with excessive, offensive lochia and a tender, subinvoluted uterus, who failed to respond to chemotherapy, underwent digital and instrumental evacuation of the uterus (curettage). Antibiotics were given to all patients before and during surgery to reduce the risk of bacteremia. In all patients treated by laparotomy/colpotomy and by evacuation of pus, surgical drains (paracolic, subphrenic, and/or pelvic) were left in situ at the completion of the operation.

Assessment of drug efficacy. The objective criteria used to compare P/S to P/C were the duration of fever after the initiation of antibiotic treatment and the number of patients who required a change in antibiotic therapy. Student's t test was used to compare the duration of fever between the two study groups. The duration of hospitalization was not considered since it was influenced by the requirement to complete 10 days of antibiotic therapy and by the type of surgery performed.

Results

Bacteriology. The types of bacteria and their frequency of isolation from PID and PPS patients were similar for both drug regimens (Table I). These similarities indicated that any differences observed between the two drug regimens probably were unrelated to bacterial etiology.

Treatment of PID. The average duration of fever in patients with PID treated with P/S was 13.22 ± 16.0 days, compared to 13.2 ± 15.1 days for those given P/C (Table II). No significant differences were found between the groups as to duration of fever and hospitalization and types of operation. Four PID patients (26.7%) treated with P/S were prescribed a different antibiotic whereas only one (11.1%) P/C-treated patient was changed to a different drug.

Treatment of puerperal sepsis. Nineteen of the PPS patients were treated with P/S and 22 with P/C regimens (Table III). Fever in patients treated with P/C was of significantly shorter duration than fever in those treated with P/S. Two of the P/S patients required a change in antibiotic treatment in contrast to none in the P/C group. No significant differences were found be-

Table II. Comparison of efficacy of P/S and P/C for the treatment of PID

	P/S	*P/C*	*Total*
No. cases	15	9	24
Days of fever ($\bar{X}$ ± SD)	13.2 ± 16.0	13.2 ± 15.1	13.8 ± 14.9
Operations			
Laparotomy	9 (60.0%)	5 (55.6%)	14
Colpotomy	5 (33.3%)	1 (11.1%)	6
Patients requiring change of antibiotics	4 (26.7%)	1 (11.1%)	5

Table III. Comparison of efficacy of P/S and P/C for the treatment of PPS

	P/S	*P/C*	*Total*
No. of cases	19	22	41
Days of fever ($\bar{X}$ ± SD)	7.5 ± 6.1	3.6 ± 5.5*	6.9 ± 8.4
Surgery			
Laparotomy	1	0	1
Curettage†	13	15	28
Patients requiring change of antibiotics	2	0	2

*$t_{39} \doteq 2.17$; $P < 0.05$.

†Evacuation of uterus.

tween the two treatment groups in age, duration of hospitalization, and the number requiring surgery.

Surgery. Twenty of the 24 PID patients had pelvic abscesses that required surgical drainage. Fourteen underwent laparotomy and six colpotomy. One of the laparotomy patients died of a pulmonary embolus on the second postoperative day while receiving P/S. None of the patients with pelvic abscess treated by laparotomy required further surgery, but two of the six patients with posterior colpotomy required laparotomy for drainage of a contralateral pelvic abscess or pelvic and paracolic abscesses during the first hospitalization. The five patients who had uncomplicated laparotomy spent an average of 16.4 days in the hospital, whereas the four patients with uncomplicated posterior colpotomy required only 9.2 days.

Over a 6 month period following discharge from the hospital, none of the study patients treated by either laparotomy or posterior colpotomy required further surgery.

Twenty-eight of the PPS patients underwent curettage or digital evacuation. One of these patients developed a pelvic abscess and peritonitis and subsequently underwent laparotomy for drainage; she died in the immediate postoperative period of septic shock while receiving P/S.

Discussion

St. Paul's Hospital is similar to other large charity hospitals in East Africa. It serves mainly poor people who have migrated from their rural homes to Addis Ababa to seek employment and a better standard of living. Employment opportunities are extremely limited, particularly for unskilled single or divorced women; they often resort to prostitution to support themselves and their children.[2] The prevalence of gonorrhea and other sexually transmitted diseases is high in this group of women.[3]

Many of the pregnant women whose infants are delivered at St. Paul's Hospital suffer from malnutrition and have little or no prenatal care. Both PID and PPS patients are reluctant to seek hospital care until they have unsuccessfully tried traditional medications or have taken antibiotics purchased from local pharmacies. Thus, they usually are seriously ill when they arrive at the hospital.

Although *Neisseria gonorrhoeae* was the organism isolated most frequently from PID and PPS patients, most cases were nongonococcal, either culture-negative (23% to 33%) or polymicrobial in etiology. Many investigators have found that nongonococcal PID patients tend to have chronic infections, respond less well to antibiotic treatment than gonococcal PID patients, and have mixed aerobic and anaerobic infections. Anaerobes can be found in abundance in 70% to 80% of PID and PPS infections.[4, 5] The frequency of isolation of anaerobic bacteria in our patients is low by comparison with other studies[4, 5] and reflects the difficulty we experienced in collecting and processing anaerobic culture specimens. The greater efficacy of the P/C over P/S for treatment of PPS may be due to chloramphenicol's acivity against anaerobes.

An advantage of the P/C regimen for treatment of PID and PPS in Ethiopia is its low cost and widespread availability. We recommend its use for the initial treatment of serious gynecologic infections, especially when bacteriologic support is not available. Other antibiotics commonly used in Europe and North America to treat serious gynecologic infections, such as kanamycin, gentamicin, or clindamycin, are too expensive for routine use in Ethiopia. These should be reserved for patients who do not improve while on the P/C regimen and who require additional antibiotics.

The outlines we devised (Figs. 1 and 2) for management of PID and PPS proved to be helpful: The PPS patients who failed to respond to antimicrobial therapy within 48 to 72 hours and who did not have pelvic abscesses responded to evacuation of products of conception or clots of blood from the uterus. We believe that delay or failure to use oxytoxic drugs to prevent retention of clots or products of conception in patients whose infants were delivered in the home or at local maternity clinics contributed directly to the development of endometritis.

As was the case in other studies, the choice and timing of operation for abdominal or pelvic abscess presented difficulties.[6, 7] Many of the patients were critically ill and could stand only minimal surgical interference. We had hoped to demonstrate the advantage of colpotomy over laparotomy in patients with well defined cystic or fluctuant cul-de-sac abscesses. Colpotomy requires only simple anesthesia, a few surgical instruments, and one assistant, in contrast to the more complex anesthesia, two or more assistants, and full set of abdominal surgical instruments needed for laparotomy. When colpotomy was successful, patients recovered more rapidly than laparotomy patients. A disadvantage of colpotomy drainage, however, may be recurrent pelvic sepsis and abscess formation.

A review of the medical records at St. Paul's Hospital from January, 1974, to September, 1975, showed that the colpotomy patients who required a repeat operation had had puerperal sepsis with peritonitis and multiple abscesses rather than a single pelvic abscess. Rubenstein and associates[8] found that 12 of the 24 patients who underwent colpotomy for drainage of a pelvic abscess and who were followed for 1 year required hysterectomy and salpingo-oophorectomy for continuing symptoms of pain, recurrent PID, or persistent pelvic masses. The only form of surgery that prevents recurrence of PID is total hysterectomy with bilateral salpingo-oophorectomy. Such surgery, however, was unacceptable to the majority of our patients who wished to retain not only their reproductive potential, but also their menstrual cycle, thereby being accepted in the Ethiopian culture as "normal" women.

We thank the nursing staff of the Clinical Research Unit and the obstetric, gynecologic, and operating room staffs of St. Paul's Hospital for their assistance; the Bacteriology and Clinical Laboratories of the Navy Medical Research Unit No. 5 for their technical support; Philippa Wilson, M.B., B.S., for abstracting the data from this study; and Craig Wallace, M.D., for his interest and encouragement.

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PREVENTION AND CONTROL

Surveillance, prevention, and control of sexually transmitted agents associated with pelvic inflammatory disease

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Control and prevention of pelvic inflammatory disease would result from successful control of *Neisseria gonorrhoeae* and genital *Chlamydia trachomatis* infections. The same control system applies to both, but while this system is well documented in gonorrhea control, there is still no evidence that it is effective for *C. trachomatis* infections. Primary prevention of PID should be realized through a reduction of the incidence of gonorrhea, and the available techniques—(1) contact tracing and epidemiologic treatment, (2) screening and case finding, (3) preventive measures, (4) health education, and (5) training of the health workers—are discussed. Secondary prevention of PID can be achieved through good management of uncomplicated infections due to *N. gonorrhoeae* and *C. trachomatis*. The approach must be adapted to local conditions and should be different in developing countries compared to countries with a specialized health care system for sexually transmitted disease. Good management of PID is crucial in preventing sequelae and most important are tracing and treating the male contacts of patients with PID. Measures of tertiary prevention attempt to rehabilitate the patient; the main sequelae after PID are ectopic pregnancy and infertility. Surgical techniques can restore tubal function, but these methods are complicated and have a low success rate. (AM. J. OBSTET. GYNECOL. 138 1064, 1980.)

CONTROL AND PREVENTION of pelvic inflammatory disease would result from successful control of those sexually transmitted agents associated with PID. Control of these agents would reduce disease incidence, thereby decreasing the number of patients at risk for PID.

The etiology of PID remains controversial.[1, 2] Until recently *Neisseria gonorrhoeae* was the only sexually transmitted agent thought to be associated with PID. Now, however, there is increasing evidence that *Chlamydia trachomatis* causes PID, including isolation of the organism and serologic changes in patients with PID and experimental infection of animals with *C. trachomatis*. *C. trachomatis* was first isolated from a patient with nongonococcal urethritis in 1964.[3] Since then genital chlamydial infections have been recognized as a sexually transmitted disease (STD) with epidemiology that is very similar to that of gonococcal infections. In men *C. trachomatis* causes urethritis and local complications such as epididymitis and prostatitis; cervicitis and Bartholin gland infection in women; and inclusion conjunctivitis and respiratory tract infection in neonates. The similarity of this clinical pattern to infections with *N. gonorrhoeae* tends to support the suggestion that *C. trachomatis* is an etiologic agent of salpingitis. The major clinical difference between chlamydial and gonococcal infections is that the former have a longer incubation period and cause milder disease.

Control of genital chlamydial infection has not been documented clearly and there is no evidence that con-

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trol measures that are effective for *N. gonorrhoeae* are also effective for *C. trachomatis*. Furthermore, throughout the world most sexually transmitted PID is related to gonorrhea. For these reasons this article will focus on gonorrhea control measures and mention only some aspects of chlamydia control efforts.

When gonorrhea is controlled, the public health problem of PID may not be solved completely. Then the importance of *C. trachomatis* and other sexually transmitted agents as causes of nongonococcal PID may become more obvious.

Primary prevention (reduction of the incidence of gonorrhea)

Clinical services which provide care for patients are the starting point for any STD control program; effective clinical services reduce the complications of STD, including PID. Clinical services alone have limited impact on control of STD, however. Available techniques for the control of gonorrhea are (1) contact tracing and epidemiologic treatment, (2) screening and case finding, (3) preventive measures, (4) health education, and (5) training of the health workers.

Contact tracing and epidemiologic treatment. For each diagnosed case of gonorrhea, at least one other exists in the community and needs treatment to prevent complications or spread of the infection. The main aim of contact tracing is to find the undiagnosed cases, thereby reducing the reservoir of infection in the community. The usual contact tracing system uses a specially trained health worker to motivate patients, elicit names, and locate contacts.[4] An alternative system uses the clinician to motivate patients who then pass referral cards to their recent sexual contacts. A study has compared both systems of contact tracing,[5] and the referral method was found to be effective but much less expensive than use of social workers for detailed interviews and field visits. Private practitioners who see the majority of patients in more industrialized countries should be encouraged to use the self-referral method of contact tracing.

All contact tracing is not equally important. Priority should be given to tracing contacts of symptomatic women, followed by symptomatic men with a repeat infection, those with a first infection, and members of high-risk groups.[6] In countries where the control organization is still weak, contact tracing can be directed initially at men with symptomatic gonococcal urethritis.

Three strategies can be followed to manage contacts of gonorrhea[7]: (1) epidemiologic treatment for all contacts, (2) treatment contingent upon Gram stain and culture results, and (3) treatment contingent upon culture results.

While the approach with empidemiologic treatment is recommended in the United States, in most STD clinics in the United Kingdom treatment of contacts is based on the results of the Gram stain and several culture tests. Most venereologists in the U. K. are against epidemiologic treatment for reasons that it may be adopted indiscriminately without examination and follow-up of the patients, and thereby may decrease standards of management. Another argument is that once treatment is administered in the absence of a firm diagnosis, the tracing of other sexual contacts becomes impossible. For the last decade an Anglo-American debate has continued regarding epidemiologic treatment.

Judson and Maltz[8] feel that periodic determination of the prevalence of gonorrhea in clearly defined groups seen in STD clinics is a rational basis for epidemiologic treatment. Wiesner[9] emphasized that decisions concerning epidemiologic treatment should also take into account (1) the probability that contacts will fail to return for visits and (2) some estimate of the risk of disease transmission based on patterns of sexual and health behavior of noncompliant infected contacts.

A default rate for infected contacts of 20% is probably the rule in most countries. If we assume that an average case of gonorrhea causes one more case in the community every 2 weeks, then one patient who "escapes" epidemiologic treatment causes 16 new gonorrhea cases in 2 months.

A decision analysis study by Johnson[7] has provided important new information on the management of gonorrhea contacts. If treatment is made contingent on the results of culture alone, 12% to 30% of female contacts and 4% to 13% of male contacts would be infected and remain untreated. Ten to forty-one percent of women and 4% to 15% of men would be treated but would receive delayed treatment. In delayed or missed treatment a risk of complications and transmission of the infection exists. These rates of delayed or missed treatment are considered unacceptable; therefore, I feel that in the United States the recommendation for epidemiologic treatment is justified. In other countries with a lower infection rate for sexual contacts, with a high validity of the laboratory tests, and with a highly reliable system for contact follow-up, another approach might be recommended. A decision analysis review such as that done by Johnson would provide essential background data for such a recommendation. In most of the developing world where facilities are less well developed than in the United States, I recommend that all contacts should receive epidemiologic treatment.[10]

What is the place of contact tracing in control of *C. trachomatis* infections?

Thirty to fifty percent of nongonococcal urethritis

(NGU) in men is due to this agent. In a recent review Bowie[11] recommended that female contacts of patients with NGU should receive epidemiologic treatment.[11] Although it has not been demonstrated that treatment of the partner diminishes the recurrence rate of NGU in male patients, the arguments are that: (1) female partners need treatment for their own benefit and that of their neonates and (2) infected women constitute a major undiagnosed and untreated reservoir of *C. trachomatis* infections. This last argument also could be applied for male partners of women with nongonococcal lower genital tract infection caused by *C. trachomatis*. At present no clear recommendations can be made in these cases.

Screening and case finding. As another outreach activity, screening and case-finding programs based on gonococcal cultures have been considered and implemented in some countries. In order to enhance the quality of discussions on the subject, common definitions should be used.[12] Screening is the testing of apparently healthy volunteers from the general population to divide them into groups according to high and low probabilities for a given disorder; the initiative is taken by the health care provider. Screening programs have been implemented for many chronic diseases. Case finding is the testing of patients for disorders that may be unrelated to their reason(s) for seeking health care. In this case the encounter is initiated by the patient. For example, an endocervical gonorrhea culture and syphilis serology in patients presenting at antenatal or family planning clinics are considered case finding.

Gonorrhea cultures should be used first as a diagnostic test in symptomatic women. This is particularly true in developing countries where the availabilitly of gonorrhea culture tests is a limiting factor.

The cost-effectiveness of both screening and case finding depends on the prevalence of gonorrhea in the group tested.

In most European countries, case-finding programs for gonorrhea in women were not implemented because cross-sectional studies repeatedly showed a prevalence rate of gonorrhea lower than 1%. In the United States, much higher prevalence rates were found, and a gonorrhea case-finding program was started in 1972. During 1977, 8.5 million women were subjected to culturing for gonorrhea, and for 393,000, cultures were positive (prevalence of 4.7%).[13] In recent years, the cost-effectiveness of this indiscriminate culture case-finding program has been questioned. Better cost-effectiveness could be obtained by concentrating culture efforts on high-risk groups, such as women attending STD clinics and hospital emergency rooms. Several attempts have been made to evaluate the effect of the case-finding program on gonorrhea control in the United States. On the basis of an epidemiologic model of gonorrhea transmission, Yorke and associates[14] concluded that, "The program seems to be holding the incidence of gonorrhea at an equilibrium, 20 percent below that which would exist without the program." Factors other than gonorrhea case finding also could be responsible for changes in incidence trends. In 1972 the United States established gonorrhea treatment recommendations. Implementation of these may have led to a decrease in treatment failure rates and gonorrhea incidence. Alternatively, sexual promiscuity could have decreased after a decade of increased promiscuity. The latter possibility is supported by observations from Sweden where gonorrhea incidence markedly declined after 1971 although case finding was not a part of the gonorrhea control program. Several factors are associated with this decrease, but of interest is a diminished sexual promiscuity since the early 1970s in Sweden.[15] Evaluation of the impact of case finding on PID incidence in the United States has led to inconclusive results, mainly owing to methodologic problems.[16–18]

Screening programs for gonorrhea should, only exceptionally, be considered due to low cost-effectiveness. Voluntary compliance is low especially in those groups with highest prevalence rates. (This is also true for other screening programs, i.e., tuberculosis and cervical carcinoma.) Screening can be considered in some high-risk groups such as prostitutes or homosexuals. Another high-risk group should include patients with gonorrhea. In addition to a test-of-cure 3 to 5 days after treatment, rescreening of such patients at regular intervals is often recommended. However, one group[19] found that the unit cost per detected gonorrhea case by rescreening 6 and 12 weeks after treatment was $796. These workers felt this activity was not cost-effective. Apart from cost-effectiveness, the control-effectiveness of screening and case-finding programs also must be considered. Control-effectiveness depends upon the proportion of existing cases missed by the program and risk of further transmission via these cases.

Serologic screening for gonorrhea is not cost-effective[20] because these tests have insufficient validity, a factor of great importance in low-prevalence situations.

Preventive methods. It is evident that methods which prevent the infection are the best tools in gonorrhea control. For most infectious diseases, primary prevention is based on manipulation of the environment or effective vaccination. STDs are related to sexual behavior, which can hardly be influenced. Available preventive methods are the so-called "prophylactic methods," which are more extensively reviewed elsewhere.[21–23]

Locally acting measures, such as use of soap and water and urination after exposure, have no effect and topically applied antiseptics and antibiotics have important side effects. Systemic chemoprophylaxis and treatment at the time of exposure prevent most gonococcal infections, but may mask more serious infections and increase the resistance of gonococcal strains in the community. Recommended personal prophylactic methods, which also can be used for contraception, are the condom and the diaphragm in combination with intravaginal capsules, jellies, and creams which have spermicidal and also some gonococcidal effect. Condoms should be highly effective in preventing the transmission of gonorrhea, trichomoniasis, and NGU. But the control-effectiveness of this method is limited owing to such factors as motivation, acceptance, and use, which differ greatly in different sociocultural settings. Nevertheless a condom campaign should be part of an STD control program. Now that the side-effects of the most widely used contraceptive methods, oral contraceptives and the intrauterine contraceptive device, are better known, the physical methods could again become more widely used as contraceptives.

A vaccine for gonorrhea is the method with the greatest control potential (except for total abstinence). Although gonorrhea does not confer natural immunity, this should not be a deterrent in the research for a vaccine. The arguments are that effective vaccines have been developed against other diseases that do not reliably confer long-lived immunity (tetanus, pertussis, and smallpox) and that the acquired infection on the genitourinary surfaces might not generate sufficient immunogenic stimulus for immunity to develop.[24]

The three cellular components (pili, proteins of the outer membrane, and lipopolysaccharides), separately or combined, are potential candidates for preparation of a vaccine.

What is the potential of a vaccine to control genital tract infections with *C. trachomatis*?

Experience with trachoma vaccines in the control of *C. trachomatis*–induced eye diseases makes this prospect rather gloomy. Reviewing the problem, Grayston and Wang[25] concluded, "It seems that control of venereal disease with *C. trachomatis* through immunization is not as promising an avenue of approach as standard public health measures." And we know from experience with gonorrhea how promising these measures are!

Health education. The role of health education in the control of STD was discussed extensively at the Technical Discussions held during the Twenty-Eighth World Health Assembly[26] in 1975. The value of health education should not be overestimated and here also priorities must be chosen. Health education aimed primarily at altering sexual behavior is not a good approach. Such education easily adopts a moralistic undertone and risks being counterproductive. Furthermore, sexual behavior depends much less on what the person knows than on what he or she prefers and values in life.

Education about STD and contraception should be integrated in sex education for children and teenagers. Education on sexuality should be part of general education. STD and unwanted pregnancy are unfortunate side effects of expressed sexuality and should be discussed as such. There is a great need for sex education in developing countries.

An editorial in an African Sunday newspaper stated recently,[27] "Grisly reports of young girls who dump, smother or bury their newborn infants are reaching immense proportions countrywide. Treating them with punishment is of no use and (is) damaging. Prevention (by sex education) is ten-tenths of the cure."

STD information should be made available to people who need it. Diffusion of STD education to the general public through advertisements in newspapers, magazines, radio, or television often results in increased demands on the health services by the "worried well." STD education should be directed toward high-risk groups. One readily identifiable group are patients with STD who present for treatment. The health education for these patients explains their disease, how to take the medication, how to present for a follow-up examination with test-of-cure, how to facilitate tracing the contacts, and how to return immediately in case of a new infection. This information is best introduced at the consultation and can be reinforced by handouts.

Training of health personnel. STD control is well established in the curricula of few medical and paramedical schools. In particular, little attention is given to public health, epidemiologic, and sociologic aspects of STD. Suggestions to improve STD medical education have been proposed.[26, 28] Special efforts in this regard have been made for the developing countries. Since 1977, the World Health Organization has organized regional workshops on STD control, with the main objective of training a team of health professionals in STD control programming. Subsequently many countries have organized national workshops and postgraduate training sessions in STD control.

Secondary prevention of PID (management of uncomplicated infections due to *N. gonorrhoeae* and *C. trachomatis*)

The health care systems for STD management differ greatly throughout the world. Some countries, such as the United Kingdom and Sweden, have well developed, specialized STD health care systems in which high-quality facilities for investigation, diagnosis, treatment, and contract tracing are widely available to the public.[29] In most industrialized countries, STD health care is provided by both public clinics and private physicians. These systems differ in patient management, case reporting, contact tracing, patient education, quality of care, use of laboratory aids, and patient fees.[30] Private physicians manage 75% to 80% of patients in the United States and more than 95% of patients in Belgium and France.

In the developing countries, health care for STD is provided by paramedical workers at the outpatient departments of hospitals, the health centers, and dispensaries. In addition many patients with STD are seen by traditional healers and other patients treat themselves. The problem of STD in the tropics differs from that in developed countries in many respects: (1) Disease incidence is five- to twentyfold greater; (2) *C. trachomatis* infections occur relatively much less frequently than gonorrhea; and (3) complications and sequelae are much more common, including PID, infertility, and ectopic pregnancies. To cope with this enormous STD problem only scarce resources are available. The annual per capita health budget in Africa rarely exceeds $5.00 and for most countries it is approximately $2.00 a year. As Arya and Lawson[31] stated, "In developing countries the management of STD is dictated by what is practical and economical to do good to the greatest number. This will necessarily fall short of the optimal measures."

In developing countries, paramedical staff of health centers have an enormous work load in meeting health care demands and addressing the health needs of the population they serve. To assist such staffs, management strategies for all diseases should be problem oriented. The health worker must identify the main health problem on the basis of presenting complaints and simple clinical examinations. For example, problems may include a fever >39° C, cough of 3 months' duration, urination of blood, etc. Uncomplicated gonorrhea and *C. trachomatis* infections are identified in a problem-oriented strategy as "urethral discharge" in men and "genital complaints" in women (dysuria, vaginal discharge, low abdominal pain). Management strategies for these problems under conditions of poor or no availability of diagnostic tests have been developed and tested in recent years.[32]

Standardization of certain aspects of disease management enhances the quality of care. The implementation of Center for Disease Control–recommended treatment schedules in the United States has improved the management of patients with gonorrhea; consequently the prevalence of gonococci less sensitive to penicillin and other antibiotics has decreased.

Management of *C. trachomatis* infections is more difficult than gonorrhea management because the diagnosis can rarely be made with certainty. There is a pressing demand by the medical profession for general availability of chlamydial culture services for routine diagnostic purposes.[33] But is such an expensive and complicated diagnostic tool really needed for acceptable case management? Urethritis in men is either gonococcal or nongonococcal, and 40% to 50% of disease in this last group is due to *C. trachomatis*. Penicillin or ampicillin is still the recommended treatment for gonorrhea in most countries. Irrespective of the isolation of *C. trachomatis*, tetracycline is still the recommended treatment for NGU. The same approach is followed in women with lower genital tract infection: Penicillin or ampicillin are recommended for gonorrhea and, again, tetracyclines (or erythromycin in pregnant patients) are recommended in cases of nongonococcal genital infection. A large percentage of patients with gonorrhea (20% to 30% of men and 35% to 65% of women) have a concomitant genital chlamydial infection. One approach is to first treat the gonorrhea and subsequently administer tetracyclines to those patients who are found to have postgonococcal genital infection. There are other possible approaches: (1) From the start treatment should be curative for both gonorrhea and chlamydial genital tract infection, for instance, 0.5 gm tetracycline four times daily for 7 days; and (2) administer single-dose effective treatment for gonorrhea in addition to tetracycline treatment for *C. trachomatis*. Controlled studies to evaluate these different approaches should allow more rational decisions in case management in the future.

Management of PID

Good management of PID, i.e., accurate diagnosis and appropriate therapy, was reviewed extensively.[34–36] Endocervical cultures have been emphasized as the method to distinguish gonococcal PID from nongonococcal PID. However, treatment recommendations are not influenced by isolation of *N. gonorrhoeae*.

The etiologic role of *C. trachomatis* in nongonococcal PID has become increasingly well documented. With declining incidence of gonorrhea, nongonococcal PID is becoming relatively more important.[37] Finally, tetracyclines are the treatment of choice for nongonococcal urethritis and nonspecific genital infection in women. All these arguments favor the use of tetracyclines (for instance, 2 gm daily for 10 days) as routine treatment for PID in outpatients, irrespective of the cause of disease. This regimen has no substantial disadvantages in cost, compliance problems, side effects, or acceptability when compared with regimens using a single dose of procaine penicillin followed by ampicillin, 2 gm daily for 10 days. Tetracycline is also effective treatment for both *N. gonorrhoeae* and *C. trachomatis* infections.

Most important for disease control is tracing and treating the contacts of patients with PID. Thirty-nine to fifty-two percent of male contacts of women with gonococcal PID and 8% to 14% of contacts of women with nongonococcal PID have gonorrhea.[34, 38] A large number of these male contacts with gonorrhea are asymptomatic (Table I), which means that compliance with follow-up can be expected to be low in this group. Sixteen percent of sexual contacts of patients with nongonococcal PID had symptomatic NGU.[34] Different approaches to management of male contacts of PID patients are possible, and the first decision is between epidemiologic treatment and treatment after making the diagnosis. The second decision concerns the type of epidemiologic treatment if only one regimen is to be used: tetracyclines (2 gm daily for 7 days) or a single dose of procaine penicillin or ampicillin. The choice between these regimens depends on the relative importance of *N. gonorrhoeae* and *C. trachomatis* infections in those male sex partners of PID patients. No study has supplied data on these infections in male contacts, but relative frequency of gonococcal PID vs. nongonococcal PID could provide some indication. Another approach could be to give epidemiologic treatment to PID contacts based on the type of PID the patient had: a single dose treatment for gonorrhea with procaine penicillin or ampicillin administered to contacts of patients with gonococcal PID and tetracycline treatment to contacts of patients with nongonococcal PID.

Tertiary prevention of PID

Measures of tertiary prevention are directed toward the rehabilitation of the patient in order to restore good health or minimize the sequelae of the disease.

Table I. Male contacts of women with acute PID*

	Gonococcal PID (no.)	*Nongonococcal PID (no.)*
Women	100	46
Contacts examined	161	69
Contacts with *N. gonorrhoeae*	63 (39%)	10 (14%)
Symptomatic	49 (78%)	7 (70%)
Asymptomatic	14 (22%)	3 (30%)

*Data from Gilstrap and associates.[38]

The main sequelae after PID are infertility and an increased risk for ectopic pregnancy. Fertility can be restored by surgical techniques but those are complicated and the rate of success is low. The chance of pregnancy is estimated at 25% but is slightly higher with use of microsurgical techniques. Surgical techniques extensively discussed in gynecology textbooks include: fimbriolysis, salpingolysis, salpingotomy, uterotubal implantation, tubal plication, resection and reanastomosis of a part of the tube, and often a combination of these interventions. A large number of examinations and investigations always precede the decision for surgery, causing considerable discomfort for the patient. A new technique for restoration of fertility recently became possible: test-tube babies. Although this technique is of great scientific value, it will not solve the public health problem of infertility in the near future.

In developing countries these solutions cannot be applied to the enormous sterility problem among many population groups. Maternity is very important in most local cultures, and in some countries mothers wear distinctive signs of their motherhood, for instance, a yellow headband. Such traditions emphasize the severe social stigma of sterility for women.

The only hope for future generations of young women is the prevention of PID, which can be achieved through the implementation of primary and secondary control measures as outlined.

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Problems in the surveillance and control of sexually transmitted agents associated with pelvic inflammatory disease in the Far East

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Little published data are available on pelvic inflammatory disease in the Far East. Measures available to control gonorrhea in the region are reviewed. The rising incidence of pencillinase-producing *Neisseria gonorrhoeae* in Singapore and the Far East and its implications concerning the pathogenesis of PID are discussed. (AM. J. OBSTET. GYNECOL. 138:1071, 1980.)

PELVIC INFLAMMATORY DISEASE has a polymicrobial etiology.[1] A substantial number of causative microbial agents are transmitted during sexual intercourse. Sexual promiscuity and permissiveness increase the risk of transmission and thus the incidence of both acute and chronic PID. PID is of interest to the venereologist, for it can be used as an index of control; a high incidence of PID is a reflection of inadequate management of sexually transmitted diseases (STDs) in the community. *Neisseria gonorrhoeae* was implicated in the pathogenesis of PID about 100 years ago, but its exact etiologic role has been much debated.[2-4] More recently, *Mycoplasma hominis*, *Ureaplasma urealyticum*,[5] and *Chlamydia trachomatis*[6] have been implicated in PID.

Considering that pelvic infection in women is often a result of either symptomatic or asymptomatic, sexually acquired infection, no physician should interest himself in treating only the individual patient but should be appreciative of the wide medical, epidemiologic, and social problems surrounding the disease. Empirical treatment not backed by sound bacteriologic diagnosis should be discouraged, especially when viewed on the basis of current data on the epidemiology and the biologic features of gonorrhea.

Very little published information is currently available on PID in the Far East. All authorities agree that untreated infection in women is one of the commonest causes of infertility in tropical countries.[7] Much of the available data comes from Africa.[8-11] Unpublished data show that acute salpingitis and chronic pelvic inflammation are not uncommon in the Far East and in the South Pacific islands.

The size and extent of the problem posed by *C. trachomatis*, *M. hominis*, and *U. urealyticum* remain undefined. Statistical reports for nonspecific urethritis in men are available for only a few countries in the Far East. In a busy public clinic in Singapore the ratio of nonspecific urethritis to gonorrhea was 1 : 15.58.[12] The impression gained from discussions with clinicians is that nonspecific urethritis is less common than gonorrhea in the region. Csonka,[13] in England, noted nonspecific urethritis to be more common in the indigenous population than in immigrants. No data are available on the role of these organisms in the pathogenesis of PID in the Far East.

Surveillance and control in the Far East

The establishment of a hexagonal approach at national and local levels is necessary to achieve good surveillance and control of gonorrhea and other STDs (Fig. 1). The approaches are: (1) an awareness of the problem at political and administrative levels to provide adequate funding; (2) a network of clinics and laboratory services manned by motivated and trained personnel; (3) accurate data collection to delineate the size of the total problem; (4) contact-tracing and case-holding facilities for good epidemiologic results; (5) identification and management of high-risk groups; and (6) detection and treatment of asymptomatic carriers of disease.

However, in developing countries this approach to control is caught in a vicious circle.[14] Fig. 2 outlines the

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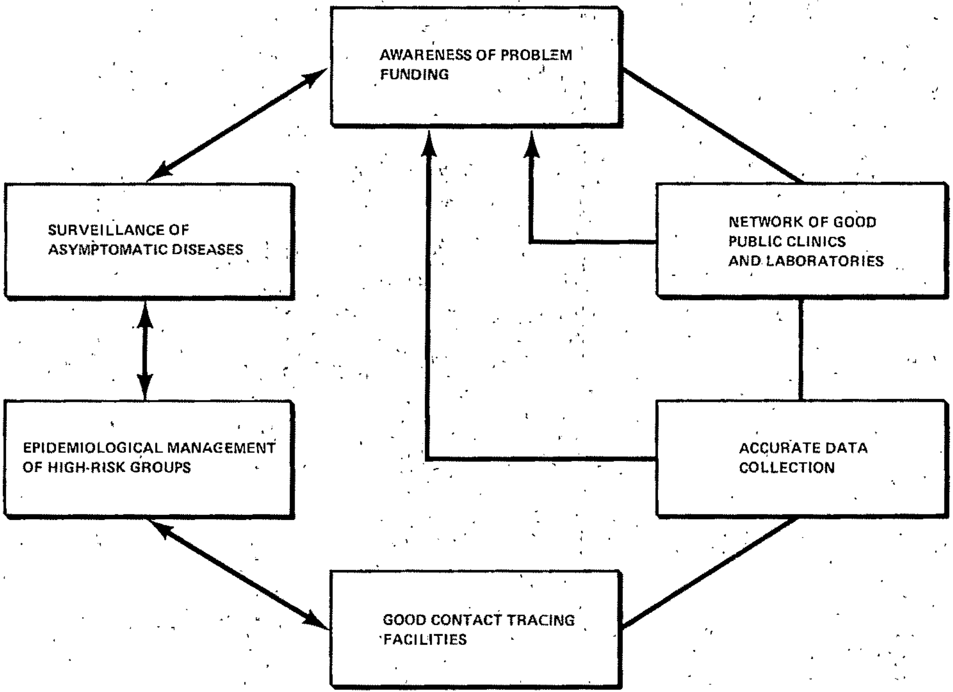
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NETWORK OF GOOD PUBLIC CLINICS AND LABORATORIES
EPIDEMIOLOGICAL MANAGEMENT OF HIGH-RISK GROUPS
ACCURATE DATA COLLECTION
GOOD CONTACT TRACING FACILITIES

Fig. 1. Hexagonal approach to good control and surveillance of STDs.

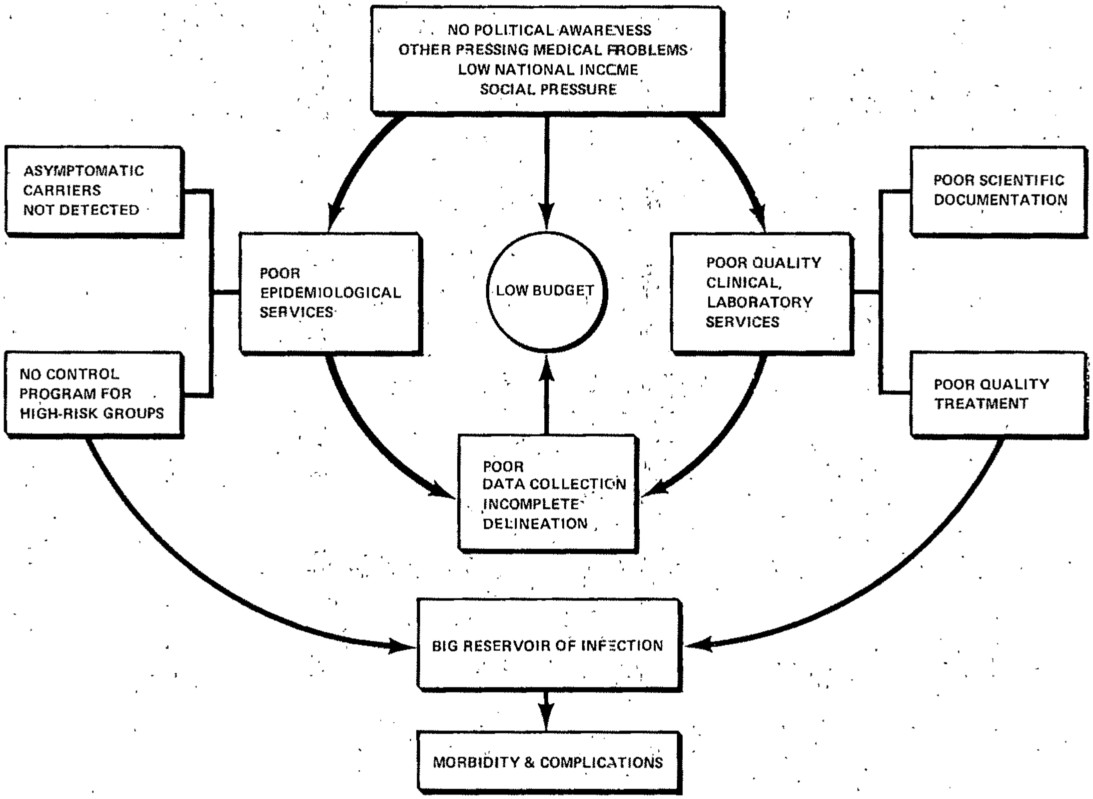
NO POLITICAL AWARENESS
OTHER PRESSING MEDICAL PROBLEMS
LOW NATIONAL INCOME
SOCIAL PRESSURE
ASYMPTOMATIC CARRIERS NOT DETECTED
POOR EPIDEMIOLOGICAL SERVICES
LOW BUDGET
POOR QUALITY CLINICAL, LABORATORY SERVICES
POOR SCIENTIFIC DOCUMENTATION
NO CONTROL PROGRAM FOR HIGH-RISK GROUPS
POOR QUALITY TREATMENT
POOR DATA COLLECTION INCOMPLETE DELINEATION
BIG RESERVOIR OF INFECTION
MORBIDITY & COMPLICATIONS

Fig. 2. Factors contributing to the vicious cycle of poor control of STDs in the Far East.

Table I. Classification of STD clinics in the Far East with reference to facilities for management of gonorrhea

	Quality of clinics		
	Good	*Intermediate*	*Poor*
Diagnosis of gonorrhea by smear only:			
Always	—	—	Most
Seldom	—	Few	—
Never	Few	—	—
Diagnosis of gonorrhea by culture (in men)			
Always	Very few	—	—
Seldom	—	Few	—
Never	—	—	Most
Diagnosis of gonorrhea by culture (in women)			
Always	Very few	—	—
Seldom	—	Few	—
Never	—	—	Most
Sensitivity studies:			
Available	Few	—	—
Not available	—	—	Most
Good follow-up to assess treatment response:			
Adequate	Few	—	—
Inadequate	—	—	Most

Table II. Classification of STD clinics in the Far East with reference to facilities for the management of nonspecific urethritis

	Quality of clinics		
	Good	*Intermediate*	*Poor*
Diagnosis of nonspecific urethritis by smears only without culture for *N. gonorrhoeae:*			
Always	Very few	—	—
Seldom	—	Few	—
Never	—	—	Most
Culture facilities:			
Available	Nil	—	—
Not available	—	One	Most
Follow-up of patients with gonorrhea to exclude postgonococcal urethritis:			
Always	Few	—	—
Seldom	—	Few	—
Never	—	—	Most

Table III. Gonococcal isolation rates in prostitutes in Singapore, by year and nationality

Prostitute group	*1977*	*1978*	*1979*
Singapore women	11.9%	11.2%	10.6%
Singapore men	7.1%	10.3%	13.0%
Thai women	—	—	14.7%
Indonesian women	—	—	50.0%

Table IV. Schemes to control STDs in prostitutes in the Far East

Quality of program	*Estimate of centers with good, intermediate, or poor programs*
Good:	
Bacteriologic diagnosis Free treatment Follow-up and assessment of treatment Retracing of defaulters	Exceptional
Intermediate:	
Diagnosis by smears Free treatment No follow-up or assessment of treatment No retracing of defaulters	Few
Poor:	
Smear diagnosis No free treatment No follow-up No retracing of defaulters	Many
No diagnosis Epidemiologic treatment No follow-up No retracing of defaulters	Many

cycle. Low budgets allocated for STD surveillance and control programs in the region lead to poor clinical, laboratory, and epidemiologic services. As a consequence, incomplete delineation of the total extent of the problem results in low national priority of such programs. This in turn allows big reservoirs of infection, leading to increased morbidity and human suffering. The nonavailability of adequate numbers of public clinics even in urban and suburban centers in the region has inadvertently driven patients to private practitioners and even quacks. This coupled with a poor diagnostic laboratory backup in the few existing public clinics has resulted in empirical rather than scientifically based bacteriologic diagnoses of gonorrhea and nonspecific urethritis. The quality of diagnosis is, in most instances, poor (Tables I and II) and has led to incorrect and inadequate treatment schedules. Poor case holding and follow-up of treated patients have made the assessment of treatment schedules difficult, if not impossible.

Few centers in the region provide adequate epidemiologic services. In most countries in the region, contact tracing, case holding, and follow-up are rarely attempted. In others, attempts to trace contacts are made through organized agencies. In Singapore, 47% of primary or source contacts and 72% of secondary or spread contacts are successfully brought in for investi-

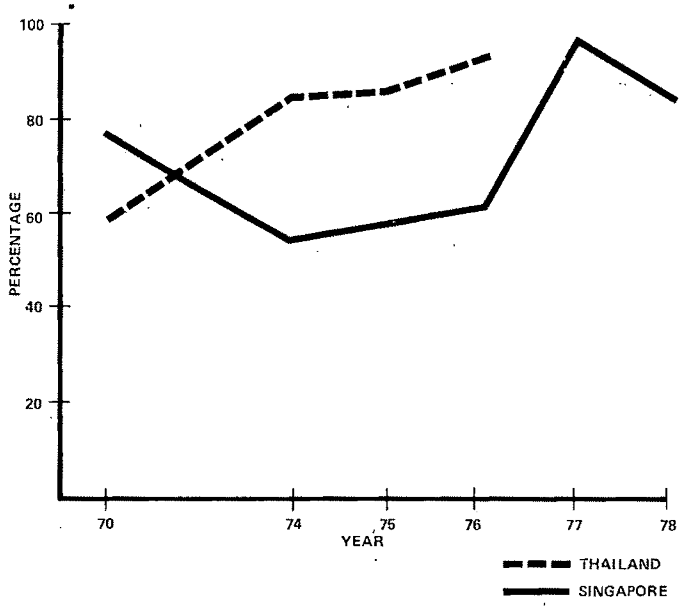

Fig. 3. Trends of partial penicillin resistance of gonococci—Singapore and Thailand.

gations. In Bangkok, the corresponding figures for 1977 were 4.9% and 78.18%, respectively.*

There are several reasons for the poor results in tracing primary or source contacts in the region. Prostitutes account for 70% to 90% of all infections in men in the Far East and Southeast Asia.[15] This is a difficult segment of the population to trace; their mobility and the lack of personal information exchanged between prostitute and client during contact are contributing factors. There is poor patient cooperation; failure to appreciate the importance of treating contacts leads to complacency. The social stigma associated with the disease forces patients to attend private clinics where provisions for contact tracing are absent.

Asymptomatic carriers

Detection of asymptomatic infections in the community is an important aspect of surveillance and control. Prompt treatment arrests complications and potential spread. Such programs provide important data on the dynamics of the disease within the community. This knowledge is an important prerequisite to implementing cost-effective control and prophylactic measures and to assessing efficiency of the measures undertaken. Yet only a few studies on this aspect are available from the region.

In an antenatal clinic in Bangkok, 11.9% of Thai women were found to have asymptomatic gonorrhea.[16] In two separate studies in Singapore, one conducted in a family planning clinic and another in an abortion clinic, the incidence of gonorrhea was found to be 1.13% and 0.6%, respectively.* The isolation rate of *N. gonorrhoeae* from a busy gynecology clinic in 1979 was 2.6% of the total examinations.*

Management of high-risk groups

Prostitutes play a pivotal role in the transmission of gonorrhea and other STDs in the Far East. They were implicated as sources of infection in 60% of sexually transmitted infections in men in Singapore in 1979. In Thailand and Indonesia, they are cited as the commonest source of infection. In the Philippines, the "hospitality girls" constitute the major source in the transmission of the disease. In the Philippines in 1969, 8.5% of 702 prostitutes had gonorrhea.[17] In a study of 200 prostitutes in Singapore in 1977, 8% were found to have positive cervical cultures and another 1% positive rectal cultures.[18] In Indonesia in 1970, 18.5% of prostitutes were found to have gonorrhea.[19] Thai and Indonesian prostitutes examined in Singapore were found to have high rates of infection (Table III). In Singapore, the female prostitutes contributed to 75% of the total cases of gonorrhea in 1979 and 77% in 1978. The gonococcal isolation rates have been declining steadily in female prostitutes from 11.9% in 1977 to 10.6% in 1979. On the other hand, in male prostitutes

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*Unpublished data.

*Sng, E. H.: Personal communication.

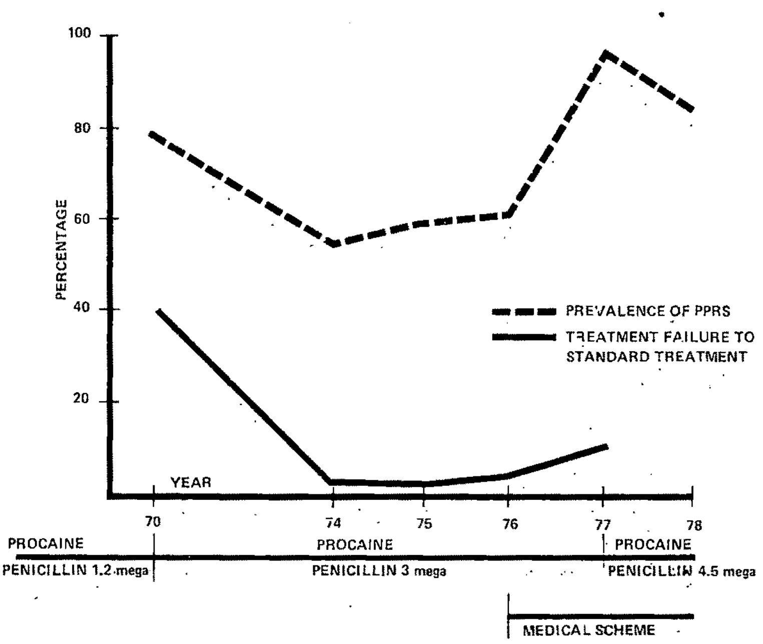

Fig. 4. Singapore gonorrhea control: trends of partial penicillin resistant strains of gonococci *(PPRS),* treatment failures on standard treatment, penicillin dose of standard treatment, and implementation of medical scheme.

the isolation rates have risen from 7.1% in 1977 to 13% in 1979 (Table III).

Recognizing the role of prostitutes in the spread of sexually transmitted infections, many countries in the region have initiated programs to control transmission by prostitutes. Individual programs vary in the degree of their sophistication and definitions (Table IV). In some of the centers, gonorrhea is diagnosed on cervical cultures and in others only by Gram-stained smears. One country in the region routinely offers prophylactic penicillin therapy every week.

In Singapore, about 65% of the total cases of gonorrhea in men occur in those between the ages of 15 and 24 years. This high-risk group is in the premarital age group and their potential for infecting their fiancées and wives can lead to medical and marital complications. However, there is some accessibility to medical treatment and health education to this group because of the compulsory national service in the Republic.

Bacteriologic surveillance

Relatively resistant gonorrhea. In the Far East antibiotics are readily available to nonmedical personnel. This has resulted in indiscriminate use and self-prescription for prophylaxis and treatment. Furthermore, the absence of monitoring facilities for bacterial sensitivities leads to the use of subtherapeutic doses by medical practitioners. This situation may be contributing directly to the high prevalence of chromosomal-mutant, resistant strains and penicillinase-producing *Neisseria gonorrhoeae* (PPNG).

The relatively resistant strains of *N. gonorrhoeae* have emerged slowly over the years and regional differences in their prevalence exist.[20, 21] In the Far East a very high proportion of *N. gonorrhoeae* strains are partially resistant to penicillin (Fig. 3). In Bangkok in 1976, 93.7% of the strains were partially resistant to penicillin. In Singapore, their prevalence has fluctuated (Fig. 4) with modification in treatment schedules. Though some centers in the region are using 4.5 megaunits of procaine penicillin or 3.5 to 4 gm ampicillin with probenecid, many continue to use smaller, subtherapeutic doses. Therefore, the resulting treatment failures of uncomplicated gonorrhea may predispose to greater risks of PID and the extent of the problem needs further study.

PPNG. The emergence of PPNG was reported simultaneously from Europe and the United States in 1976.[22, 23] The strains were speculated to have originated in West Africa and the Far East, respectively. The strain was reported from Singapore in 1977.[24] Since then, it has been isolated in at least nine Far Eastern countries[25] and has become endemic.

Table V lists the number of cases of gonorrhea in prostitutes and the general public in Singapore. The

Table V. Total number and percentage of PPNG and non-PPNG cases, by sex, in Singapore (1976-79)

Yr	*No. in men*		*No. in women*		*Total*	
	Non-PPNG	*PPNG*	*Non-PPNG*	*PPNG*	*Non-PPNG*	*PPNG*
1976	2,212 (99.94%)	1 (0.05%)	2,016 (99.91%)	2 (0.09%)	4.073 (99.73%)	3 (0.07%)
1977	1,833 (99.57%)	8 (0.43%)	5,813 (99.76%)	14 (0.24%)	7.646 (99.71%)	22 (0.29%)
1978	1,822 (89.0%)	220 (11.0%)	6,214 (89.0%)	796 (11.0%)	8,036 (89.0%)	1,016 (11.0%)
1979	1,835 (81.0%)	430 (19.0%)	5,714 (81.0%)	1,362 (19.0%)	7.540 (81.0%)	1,792 (19.0%)

Table VI. Percentage of PPNG in female prostitutes and public in Singapore (1976-79)

Yr	*% PPNG*	
	Prostitutes	*Public*
1976	0.11	0
1977	0.19	1.30
1978	11.33	11.81
1979	18.79	24.6

Table VII. Cases of gonococcal ophthalmia neonatorum and rate per 100 live births in Singapore (1975-79)

	1975	*1976*	*1977*	*1978*	*1979*
No. of cases	77	44	20	22	13
No./100 live births	0.19	0.01	0.05	0.05	0.03
No. with PPNG-induced ophthalmia neonatorum	0	0	0	3	1

steady increase in the incidence of infection in women and men can be attributed to better facilities for case detection provided by the epidemiologic control programs started in 1976 for prostitutes. With the successful implementation of the epidemiologic control programs, they are an easily accessible group for control.

The incidence of isolation of PPNG has been increasing steadily since 1976, when the first three cases were isolated in Singapore (Table V). In 1977, 22 strains were isolated (sevenfold increase); in 1978, 1,016 strains (fiftyfold increase), and in 1979, 1,868.

The rise of PPNG has been significant over the past 4 years despite control measures, bacteriologic monitoring, and use of effective antibiotics. At Middle Road Hospital, we have been using 2 gm kanamycin intramuscularly as standard treatment for these strains, with a failure rate of 2%.[26] Significantly, the incidence of non-PPNG strains dropped from 8,036 in 1978 to 7,464 in 1979 ($p < 0.01$). This pattern suggests a shift of the bacterial sensitivity.

Table V shows a breakdown in the incidence of PPNG and non-PPNG in men and women. In men, incidence of PPNG has risen from 0.06% in 1976 to 18.7% in 1979 and in women, from 0.09% in 1976 to 19% in 1979. There also has been a corresponding drop in the incidence of non-PPNG strains in both sexes from 1978 to 1979.

It has been erroneously inferred that PPNG strains were confined to the female prostitute.[27] Table VI shows the distribution of PPNG infections in female prostitutes and the general public. The distribution and rise in both groups paralleled each other until 1978, but in 1979, the rate of increase was higher in the general public. If these trends are maintained a greater increase in PPNG-induced ophthalmia neonatorum and PPNG-induced pelvic infections can be predicted.

The first case of PPNG-induced ophthalmia neonatorum was reported in 1979 from Singapore.[28] Since then, more cases of ophthalmia neonatorum have been recorded (Table VII). It is disturbing to note that for the first quarter of 1980, three cases have been recorded. There are no reliable data for the role of PPNG in acute and chronic PID in Singapore. However, in report of a pilot study of 25 cases of gonorrhea in 1 year at an obstetric and gynecologic hospital, PPNG strains were detected in 16% of the cases. One patient with this strain had PID.[29]

The clinical significance in the shift of bacterial sensitivity from penicillin-susceptible to penicillin-resistant strains in the Far East is far-reaching. A decision to change the standard treatment from penicillin to an aminoglycoside may have to be made soon. This is especially relevant in gynecologic practice where it is standard procedure to treat pelvic inflammation with ampicillin and tetracycline. Our experience in Singapore emphasizes the importance and need for a complete bacteriologic diagnosis before embarking on treatment.

Conclusion

The control and surveillance of agents contributing to the pathogenesis of PID in the Far East leave much to be desired. Although the ill effects of this lack of control is still an "iceberg," ugly facts are emerging in clinical practice. Unless control programs are adequately budgeted, planned, and run with more efficiency and vigor, the problems can only worsen.

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Problems and proposals for the surveillance and control of sexually transmitted diseases associated with pelvic inflammatory disease in South America

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In South America, the number of women at risk for gonococcal pelvic inflammatory disease has increased greatly along with the incidence rates for gonorrhea. Further information is needed on the epidemiologic aspects of gonorrhea and PID in order to develop programs for optimal control of sexually transmitted disease (STD). We think that the control of STD, including PID, requires improvement of four basic factors: professional training in STD, management of STD by all levels of health care services, laboratory diagnostic services, and public education in STD. (AM. J. OBSTET. GYNECOL. 138:1078, 1980.)

IN SOUTH AMERICA, the reported incidence of sexually transmitted diseases (STDs) associated with pelvic inflammatory disease has increased greatly. Despite increases in the incidence of gonococcal infections, the magnitude of the gonococcal PID problem, its epidemiology, and its impact on the health of women are largely unknown. Information on PID associated with other STDs is virtually nonexistent. In order to summarize the problems encountered in the control of STD agents associated with PID, I reviewed official documents prepared for the Pan American Health Organization, as well as articles published in journals of South American obstetric and gynecologic societies, and I have drawn on personal experience.

Control problems

The problem of PID is largely one that involves fertile women between the ages of 15 and 49 years. These women are now at higher risk for STD, and therefore PID, than ever before, presumably because of the earlier onset of sexual activity, more permissive attitudes of society, and medical advances that have reduced fear of the consequences of sexual activity, namely, venereal diseases and unwanted pregnancy.

Not only are fertile women at greater risk for STD, but the number of such women has increased greatly (Table I). By the year 2,000 A.D., it is estimated that the number of women in the age group 15 to 49 years will have increased by 280% over the number in South America in 1950 and by 80% in North America. The prediction is that the tropical countries of South America (Bolivia, Brazil, Colombia, Equador, Paraguay, Peru, Venezuela) will have an increase in fertile-age women of 347% during this period, whereas in the temperate countries (Argentina, Chile, Uruguay) the increase will be 105%. These increases mean that many more women will be at risk for PID if the STD epidemic continues.

Between 1970 and 1975, reported incidence rates of gonococcal infection increased 29% in Bolivia, 25% in Uruguay, 15% in Argentina, and 13% in Colombia (Table II). In contrast, the rates declined in Paraguay by nearly 50%. The largest increase in the Americas during this period was in the United States, with a 62% increase.

The surveillance systems for STD are inaccurate in countries of South America. First, few of the total number of cases are reported. No less than 70% of infected patients seek care from private practitioners; these physicians virtually never report cases because they fail to appreciate the importance of STD. Moreover, the diversity of institutions and the fragmentation of medical care are barriers to the establishment of surveillance systems. Diagnostic accuracy is poor even for reported cases because professionals are in-

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adequately trained and diagnostic tests are unavailable. Finally, relevant analysis of the surveillance data is not performed, reports are delayed, and professionals do not receive relevant information. Therefore, the data that are collected are not used optimally, and opportunities to encourage improved reporting by physicians or to enhance administrators' attention to the STD problem are missed. For example, although the association of gonorrhea and PID is well known, essentially no epidemiologic information on PID has appeared in recent issues of South American medical journals.

Health care services in South America provide highly variable population coverage. In all countries, health authorities recognize the need to extend health care coverage to the sizable population groups that are not currently covered for geographic, cultural, functional, or financial reasons. Coverage for STD problems is as inadequate as it is for general health problems.

The network of diagnostic laboratories is inadequate. As a result, diagnostic tests are ordered infrequently, and more demanding tests, such as cultures, which are essential for the diagnosis of gonorrhea in women, are rarely utilized. Finally, even when diagnostic tests are sought, specimen collection is poorly carried out, thus producing inaccurate findings. At present, 50% of the medical facilities to which physicians are assigned are not capable of performing a Gram stain smear. The Ten Year Health Plan for the Americas recommended the development of health laboratory systems which would ensure that minimum laboratory services are available to physicians in medical facilities.

STD control programs

In most countries of South America, programs for the control of STD are limited to the control of prostitution and evaluation and treatment of prostitutes, prenatal serologic screening for syphilis, and studies performed in women attending hospital gynecology clinics. Because epidemiologic services are underdeveloped, systematic control and surveillance strategies are lacking. I think that existing STD control programs are incomplete and have minimal impact on disease control. However, other factors contribute to poor STD control, as follows.

Medical care system. Medical care in most South American countries is characterized by institutional diversity, with little coordination between institutions. Because of this diversity, information systems are different and clinical services are highly variable in the problems emphasized and the quality of care. Health systems also heavily emphasize care for acute illnesses rather than preventive health care. This situation inhibits the development of standard criteria for diagnosis and management of STD cases, standardized reporting systems, and a prevention focus for STD control.

Table I. Number of women aged 15 to 49 years in the Americas, 1950 to 2000*

	No. (millions) per year					
	1950	*1960*	*1970*	*1980*	*1990*	*2000*
North America	42.4	45.9	54.1	63.8	70.7	76.1
Tropical South America	20.1	26.3	35.5	48.5	65.9	87.9
Temperate South America	6.5	7.7	8.9	10.2	11.7	13.3

*Data from The Health Conditions in the Americas, 1973-1976, Pan American Health Organization/World Health Organization Scientific Publication No. 364, 1978.

Medical care coverage. Not only are some groups inadequately provided for by the health care system, but the care is fragmented. Thus, preventive health care needs, minor acute illnesses, and illnesses that require hospitalization generally are provided by distinct institutions.

Professional STD training. The training of medical personnel in diagnostic, treatment, and control aspects of STD is inadequate. These diseases are given little attention in curricula for undergraduates. Consequently, graduates are not prepared to diagnose and manage the growing number of infected patients seen in public and private clinics.

Laboratory system. The inadequacy of the laboratory network prevents accurate diagnosis and management of cases, case finding, and epidemiologic surveys. This deficiency accentuates other problems of the health care system for STD.

Antibiotic misuse. Antibiotics are readily available in virtually all countries, and self-medication with inadequate doses of antibiotics is common. The problem of inadequate self-medication is particularly common in rural areas of many South American countries.

Public education. The public seems unaware of the severity of STDs and their complications, and also lacks information about the prevention, treatment, and control of these diseases.

Proposals

Although systems for reporting STDs are inadequate in South America, I am confident that STDs represent a real public health problem. As in the rest of the world this problem is growing rapidly because of so-

Table II. Annual incidence rates of gonococcal infections per 100,000 population in selected countries of the Americas, 1970-75*

Country	Year						% Change
	1970	1971	1972	1973	1974	1975	
Argentina	44.1	—†	73.8	77.6	69.8	52.5	+15.0
Bolivia	24.2	38.7	33.0	34.3	—	—	+29.0
Colombia	182.5	165.2	164.3	188.0	193.3	207.7	+13.0
United States	289.8	323.7	367.4	400.5	427.2	477.0	+62.0
Paraguay	39.6	29.8	28.3	26.0	—	20.0	−49.0
Uruguay	9.0	18.2	21.3	21.0	12.0	—	+25.0

*Data from World Health Statistical Annual, vol. II, Geneva 1970, 1971, 1972, 1973, World Health Organization, 1976.
†Dashes indicate no data available.

cial, cultural, educational, and medical factors. We must define the magnitude of the STD problem and characterize the epidemiology of gonorrhea and PID in order to adopt the most effective strategies for their control. Control programs must be adapted for and integrated into the health care delivery system of each country.

I think that ignorance about STD on the part of physicians is a major barrier to more effective control programs. Thus, STDs are underreported and mismanaged, and their consequences ignored. Courses which deal with STD must be incorporated once again into medical curricula for undergraduate and postgraduate students, being taught as an integral part of general medicine and obstetrics and gynecology but also incorporated into other specialties, such as urology and dermatology. Emphasis should be placed on learning standardized diagnostic and treatment techniques. However, this training should be supplemented by information on disease epidemiology and control in order to ensure adequate training of physicians. Improved professional training will ensure effective management of symptomatic patients who attend health care facilities and make less difficult case-finding activities among other groups of patients at risk for disease.

Clinical services for the STD must be available for the majority of a nation's population. Some specialized venereal disease clinics exist in South America, but these are focused on prostitute populations. Although STD specialists and special clinics have an important role in national efforts to control sexually transmitted disease, care for patients with STD problems must be available from all physicians if control is to be effective. Since the social security systems provide health care coverage for nearly 50% of the population in most countries, I think that implementation of STD control in primary health care facilities should begin with the social security health system. After the establishment of diagnostic, treatment, and management norms in the social security system, these elements of control can be extended to other primary health care providers. In this way, control coverage of STD eventually could extend to the entire population.

STD control has been established in the social security health care system of Mar Del Plata, Argentina. Planning for this control program was undertaken jointly by the city's social security system and the National Institute of Epidemiology under the State Secretary of Health. The initial phase has been the development of a case-finding system to determine the incidence of STD among the population served. In order to obtain this information, it was essential to strengthen the laboratory network with training, personnel, and facilities. Building on the experiences gained in this joint effort, it has been possible to undertake national STD control efforts. We think that the experience in Argentina may be of value to other programs in South American countries.

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Problems and approaches in the surveillance and control of sexually transmitted diseases: The Cuban experience

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Cuba has a comprehensive National Health System which delivers primary and secondary health care to 100% of the population. Communicable disease control and preventive medicine programs are implemented through this System. The National Syphilis Control Program is described briefly as an example of a successful control program for sexually transmitted disease. Data defining the magnitude of the problems of gonorrhea and pelvic inflammatory disease are not complete. A program for improved gonorrhea surveillance and control is just beginning. Further improvements and refinements in this program are described. (AM. J. OBSTET. GYNECOL. 138:1082, 1980.)

CUBA IS A COUNTRY situated in the Caribbean region. In 1959, true economic development began, and as a result, public health care became a state responsibility.

The organization of a National Health System has permitted implementation of successful activities to control and eradicate the principal communicable diseases affecting human health.

Primary health care is provided by the polyclinics, where specialized medical services in pediatrics, internal medicine, gynecology-obstetrics, and dentistry, as well as x-ray and clinical laboratory facilities, are available (Fig. 1). A secondary level of health care has developed parallel to the primary level and consists of hospitals offering health care in all medical and surgical specialties. This preventive-clinical network guarantees coverage for the entire population. Each polyclinic offers regionalized medical care to an average of 30,000 inhabitants, and each hospital to approximately 250,000.

In 1962, the First National Forum on Hygiene and Epidemiology outlined the basis for a syphilis control group for Cuba. This program was drawn up along very general lines and was the first step toward awareness of epidemiologic data. In 1972, as the result of an analysis of the program during the previous 10 years, a new program was prepared, radically changing the philosophy of the work being carried out against syphilis. The new program emphasized an active search for cases of the disease, backed up by an epidemiologic analysis of those cases detected. Additional measures included the following nine:

1. Blood testing techniques were standardized. The VDRL* test was selected as the sole serologic test.
2. Graduate nurses were trained in the techniques of epidemiologic interviewing and were assigned to each polyclinic exclusively for this task on a full-time basis.
3. Medical personnel (dermatologists, epidemiologists, obstetricians, urologists, clinical laboratory technicians, psychologists, sanitary educators, etc.) were fully briefed according to their specialities, during national and provincial seminars on the new programs.
4. The venereal disease control program was totally integrated into the general health services.

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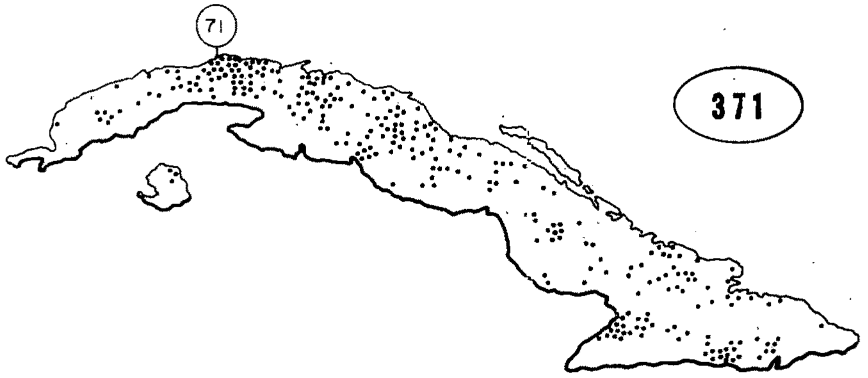
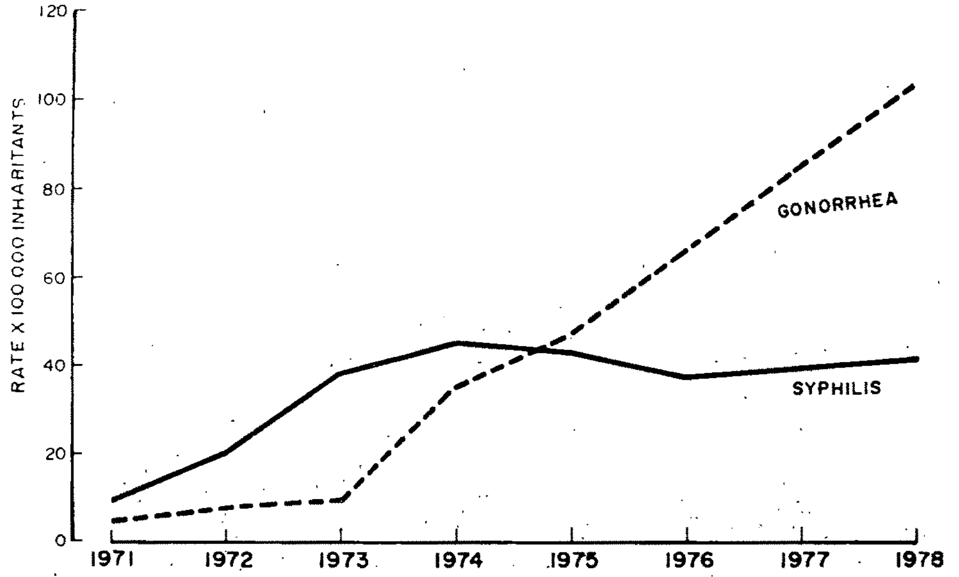


Fig. 1. Location of primary care polyclinics in Cuba, December, 1978.



	1971	1972	1973	1974	1975	1976	1977	1978
SYPHILIS	8,8	19,9	37,7	45,1	42,9	37,2	39,3	41,3
GONORRHEA	4,3	8,0	9,6	35,2	47,0	65,8	85,8	103,5

SOURCE: Provincial Depts. of Epidemiology

Fig. 2. Reported incidence rates of early syphilis and gonorrhea per 100,000 inhabitants, Cuba, 1971-78.

5. Treatment with adequate follow-up of every diagnosed case was established.

6. Diagnostic criteria were clearly defined.

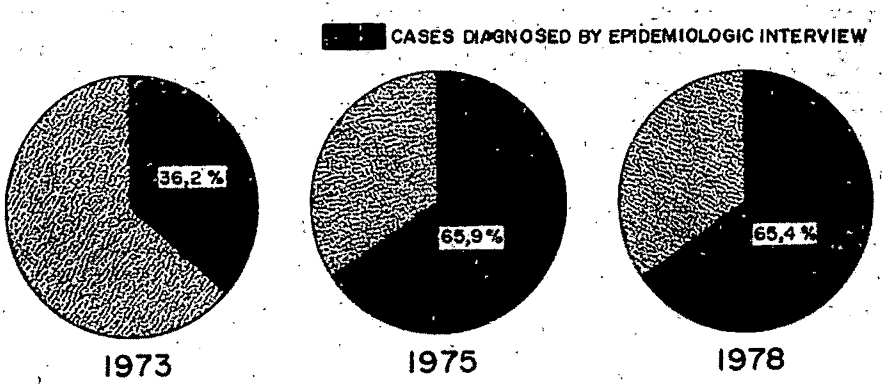
7. The prevention of congenital syphilis was assigned the highest priority within the control program.

8. An active search for cases was carried out by: (a) testing of sources of infection (location and examination of contacts, suspects, and associates), (b) study of groups considered at high risk, (c) preventive health examinations, and (d) routine serologic testing (for blood donors, outpatients, hospital inpatients, etc.).

9. The prophylactic treatment of all contacts and a subsequent checkup were instituted, as well as the study of identified suspects and associates.

The implementation of the program adopted in 1972 yielded much more exact figures on the behavior of this disease in recent years.

The success of the Syphilis Control Program is reflected by our statistics. Although the incidence rate of syphilis stabilized at approximately 40 cases per 100,000 population (Fig. 2), over 98% of all reported cases of early syphilis were completely treated and followed up. Interviewing and contact-tracing techniques improved (Table I), and 25% and 30% of the contacts



SOURCE: Provincial Depts. of Epidemiology

Fig. 3. Percentage of cases of early syphilis diagnosed by epidemiologic interview, Cuba, 1973, 1975, and 1978.

Table I. Early syphilis: percentage of interviewed patients referred to total cases reported, Cuba, 1973, 1975, and 1978*

Year	*Percentage*
1973	93.2
1975	96.4
1978	97.4

*Data from Provincial Departments of Epidemiology.

Table II. Early congenital syphilis: cases reported, Cuba, various years*

Year	*No. of cases*
1973	17
1975	8
1978	8
1979	7

*Data from Provincial Departments of Epidemiology.

and the suspects/associates, respectively, were diagnosed as having syphilis. Fig. 3 demonstrates the increase in detection of early syphilis cases as a result of epidemiologic interviews. Serologic testing on at least two occasions during prenatal care has reduced cases of congenital syphilis to a minimum (Table II).

With regard to syphilis, and by way of summarizing, we can safely say that Cuba has a fairly precise idea of the magnitude of the syphilis problem since the number of cases reported comprises between 90% and 95% of the true incidence. The complications of this disease have been reduced to a minimum, as shown by the small number of cases of late and congenital syphilis.

The development of our National Health System and the coverage now achieved have controlled the syphilis problem. This is attributed basically to the profundity of the epidemiologic work.

If we can state that our Syphilis Control Program is well developed, the same is not true for programs for gonorrhea and its most frequent complication, pelvic inflammatory disease. The number of reported cases of gonorrhea has been increasing by more than 30% yearly (Table III). The rate in 1979 was 137.8 cases per 100,000 inhabitants.

Most of the reported cases (95%) are in men. This situation has led to the preparation of a control program to be carried out progressively for the purpose of discovering the frequency of gonococcal infections among women in order to institute prompt and adequate treatment and control complications.

In the planning and implementation of this control program, we have had to face several questions (six to follow) for which we must find answers, bearing in mind not only the clinical and epidemiologic characteristics of gonorrhea but also the present availability of human and material resources in different fields:

1. We must determine the magnitude not only of gonorrhea infections but of their complications among women, and determine the number of cases among men with greater accuracy.
2. The programs eventually must cover the entire population on a progressive basis.
3. To this end, mechanisms must be set up to permit prompt diagnosis and timely notification.
4. A form of treatment considered rapid and effective has been adopted.
5. Epidemiologic investigations will be aimed at guaranteeing the rational utilization of interviewing personnel.
6. The results expected are an increase in the total

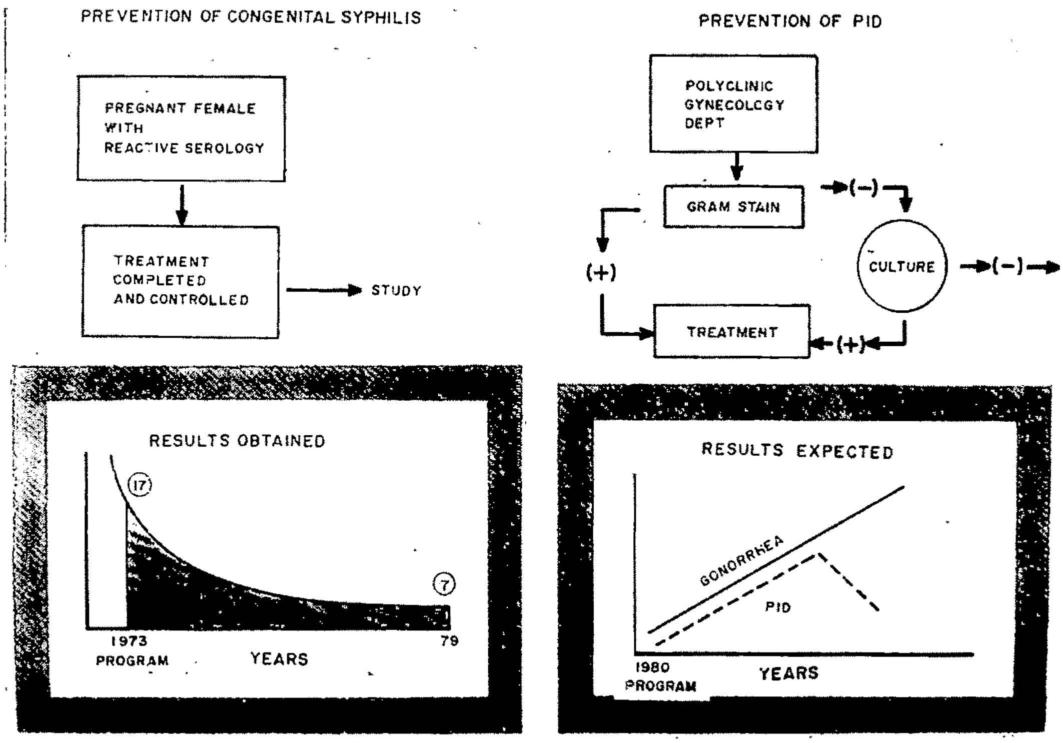


Fig. 4. Model for prevention and control of congenital syphilis and PID.

Table III. Gonorrhea: cases reported and percentage increase of notification over previous year, Cuba, 1975-79*

Yr	*No. cases reported*	*Increase in notification (%)*
1975	4,385	—
1976	6,229	42.0
1977	8,239	32.2
1978	10,211	23.9
1979	13,705	34.2

*Data from Provincial Departments of Epidemiology.

number of cases detected, as well as an increase in the percentage of cases among women.

The first stage of the program calls for the development of those means which will allow the detection of the greatest possible number of cases of gonorrhea among women. This will prevent the appearance of its most important complication, PID.

Steps similar to those adopted for the prevention of congenital syphilis will be taken (Fig. 4).

What are some of the measures formulated thus far?

1. We must utilize direct diagnosis by means of the Gram stain in our polyclinics so as to identify correctly intracellular gram-negative diplococci.

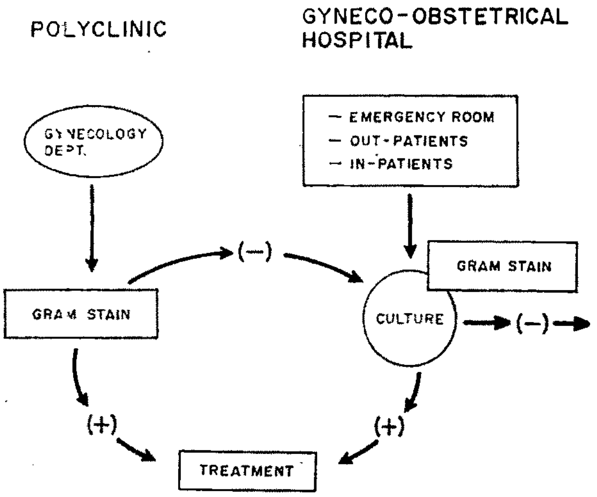


Fig. 5. Model for the prevention and control of gonorrhea.

2. We must develop in our gynecologic-obstetric hospitals the capability to diagnose *Neisseria gonorrhoeae* by culture so as to confirm the results of the direct tests (a quality-control function), and we must take samples from those women who present suspicious symptoms but have negative results on the Gram stain test.

3. Standard first-line treatment will include a dose of 5 megaunits of procaine penicillin G with 1 gm pro-

benecid, or 3.5 gm ampicillin plus 1 gm probenecid. In cases of allergy, 10 gm tetracycline will be administered over a period of 5 days.

4. The activity of the nurse interviewers will be aimed primarily at the investigation of contacts of men to detect infected women.

5. In the first stage, the search will be directed at women who present with symptoms, and later, as resources permit, it will be widened to include asymptomatic women (Fig. 5).

6. The first stage in the development of the program will involve selected polyclinics and gynecologic-obstetric hospitals; later on, it will be extended to all our primary health care units and will provide coverage in keeping with our National Health System.

7. Medical personnel, both clinical and laboratory, will be trained in order to guarantee the qualitative development of the program.

At present, different aspects of the program, as well as the gathering of PID-related information, have begun in different health care units throughout the nation.

As an example, we may cite the work being carried out at the Ramón González Coro Gyneco-Obstetrical Hospital in the city of Havana. Nine polyclinics refer patients for secondary health care to this unit, which serves about 270,000 inhabitants.

General statistical data for 1979 related to this program are as follows: (1) number of beds = 259, (2) patients discharged = 17,724, (3) patients discharged from gynecology services = 4,163, (4) patients with PID admitted = 160, (5) cases of puerperal sepsis = 91, (6) septic abortions = 11, (7) new referred cases of infertility = 54, and (8) ectopic pregnancies = 37. Figures for the past 5 years have remained at similar levels.

The diagnosis of PID is made through clinical examination, including checking of temperature and pulse, abdominal and gynecologic examination, laparoscopy, laparotomy, and colpocentesis, as well as erythrocyte sedimentation tests.

Gynecologic referrals, including those concerning contraceptives and infertility, as well as spontaneous and induced abortions, provide a starting point in the search for asymptomatic gonorrhea among the population of the nine polyclinics.

The microbiologic study of 1,331 symptomatic patients, using Thayer-Martin culture medium, resulted in a total of 9.4% with cultures positive for gonococci. Endocervical smears confirmed a high (?) rate of positive results. A randomized investigation of 295 asymptomatic women resulted in a positive culture rate of 1%.

The data presented in this article summarize the development of activities for the surveillance and control of sexually transmitted diseases as carried out in Cuba. We have placed special emphasis on the problem of syphilis control inasmuch as we can provide data from several years' work.

In the control of gonorrhea and PID, its most frequent complication, we have described the fundamental approaches that we are adopting on the basis of the study and analysis of worldwide experiences in this field. These approaches are being adapted to our National Health System to guarantee medical attention for our population on a level with present scientific and technical progress.

The authors express their indebtedness to Caridad Almanza, M.D., for her assistance in the microbiologic aspects of this work.

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Problems and approaches in the surveillance and control of sexually transmitted agents associated with pelvic inflammatory disease in Africa

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The problems in surveillance of sexually transmitted disease (STD) in Africa are numerous and are based mainly on socioeconomic barriers. Policies for controlling STD are either inadequate or nonexistent in many African countries. Gonococci are becoming increasingly insusceptible to penicillin and other drugs because of widespread use of inadequate therapy. Gonorrhea is recognized as the major pathogen of pelvic inflammatory disease in Africa. The magnitude of the PID problem is so great that current efforts are directed at case treatment rather than surveillance and control. The formation of the African Union Against Venereal Diseases and Treponematoses is an important step, and its suggestions and resolutions form a sound basis for improved STD control in Africa. (AM. J. OBSTET. GYNECOL. 138:1088, 1980.)

INSOFAR AS AFRICA is concerned, the key word associated with control of pelvic inflammatory disease is "problems." When problems are the major focus, then the approaches to surveillance and control are limited.

PID is the most common gynecologic problem in many parts of Africa. About 40% of acute admissions to the gynecology wards of two Nairobi hospitals are due to PID.[1] The same has been observed in Rhodesia, where 44% of all gynecologic admissions were due to PID.[2] It is certain that many other developing African countries have similar situations.

The causative agents of PID have been fairly well delineated in many centers throughout the world. The main sexually transmitted agents are gonococci, chlamydiae, and mycoplasmas; non–sexually transmitted agents include anaerobes and, to a small extent, some aerobes. I will consider only the first category.

The gonococcus is the major pathogen in PID in Africa.[1-3] Among 58 consecutive women with PID seen in Nairobi, 43% had cultures that were positive for gonorrhea, and an additional 10% had positive smears but negative cultures.[1] In Kampala, Uganda, of all women with PID, 38.3% had positive cultures.[3] In the Rhodesian study, gonorrhea was isolated in only 4% of women with PID.[2] There is, however, general agreement by all three centers that the isolation rate of gonococci from patients with PID would have been higher if some patients had not received antibiotics before the studies were conducted. Because this is an acutely painful condition, the common practice is for patients to receive an injection of antibiotic when they seek care at primary health care centers. After this injection of antibiotic, the patients are referred to large hospitals where cultures can be obtained.

In Africa there has been little study of other sexually transmitted agents associated with PID. There are multiple reasons for this neglect, but two general problems can be mentioned. (1) There are too few competent gynecologists to cope with the therapeutic, surgical, epidemiologic, and research aspects of the enormous PID problem. Therefore, the doctors tend to focus on urgent therapeutic and surgical management problems and to neglect the epidemiologic and research aspects Moreover, since few workers appreciate the seriousness of the PID problem, they fail to accord it adequate attention over and above their many other duties. (2) There is a general shortage of laboratory support because of inadequate facilities and a scarcity of laboratory manpower. Additionally, except for gonococci, other sexually transmitted agents responsible for PID are difficult to isolate because of the requirement of sophisticated laboratory equipment and supplies and the use of procedures which are not available in most African laboratories. Since we are still groping in the dark so to speak, for the causative agents of PID, it is difficult to discuss surveillance and control of them in Africa.

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Surveillance

Surveillance is based on the finding of cases. Active case finding in a rural community is difficult and tedious. Arya and associates,[4] working in two Ugandan rural communities, showed that in one district with a low fertility rate, as many as 18.3% of all the women examined were infected by gonococci, whereas the infection rate was much lower (2.3%) in another district with a high fertility rate. However, the resources and manpower available for health work make such community surveillance on a continuing basis practically infeasible.

Active case finding in urban areas is equally difficult. Although small, limited surveys are conducted occasionally, these are inadequate as a surveillance system. Preemployment examination for sexually transmitted disease (STD) is conducted for certain job groups in some countries in Africa. Before they can begin to work, women employed in beer bars, night clubs, and some restaurants are supposed to produce certificates that indicate that they are free of STD. Moreover, these women are supposed to be examined regularly and treated if STD is identified. In countries in which prostitution is recognized, although not legalized, the law is that if any of the prostitutes are found to harbor any STDs, they are liable to a fine or imprisonment, or both. Occasionally, the police round up these women and present them to doctors for examination. In the absence of a doctor, the women usually are interrogated by the policemen. The women usually admit their guilt to the policemen and are then taken before a magistrate for fine or imprisonment. Since these women are a large reservoir for STD, transmit much of this type of disease, and are potential candidates for PID, it is important that these health examinations be done more effectively. Greater support from many authorities will be necessary to improve these practices in Africa.

Because active case finding has so many shortcomings, passive case finding remains the main method of discovering women who are infected with sexually transmitted agents of PID. These women are identified and a diagnosis is made when they come forward because of symptoms. Proportionately, the number of infected women discovered by the passive method of case finding is small since most women are asymptomatic. Asymptomatic infected women also perpetuate the STD problem because they are unaware of the infection. Even infected women in whom a diagnosis is made can rarely name or bring their contacts for treatment. In fact, an attempt at tracing contacts frightens the contacts and drives them to private doctors. Because these patients can ill afford the fees for appropriate private care, they often receive inadequate investigation and treatment.

A number of sexually transmitted agents are also passively identified by testing women who attend family planning clinics in Africa.[5-8] The gonococcal infection rate in these clinics ranges between 2% in Swaziland and 19.5% in Nairobi, Kenya. Investigations to discover sexually transmitted agents are not routine since family planning clinics lack adequate laboratory facilities. Therefore, many women who attend these clinics will have an intrauterine contraceptive device (IUD) inserted through the gonococcal infection, thus compounding the severity of the infection and resulting in PID. In a few patients with a florid form of STD the condition will be recognized and treated before insertion of any IUD, thus preventing possible PID. The invitations to partners, i.e., husbands, to attend the clinic for investigations and treatment are easier and more acceptable for women attending family planning clinics than for those attending other clinics for their STD problems.

Other problems which impede surveillance of STD in Africa are mainly socioeconomic. In performing medical surveillance of any type in Africa, there are difficulties owing to geography and communication. Funds and manpower are inadequate to support coverage for the areas one would wish to tackle. In some areas, ethnic and tribal barriers hamper STD surveillance. In Uganda, these factors made it difficult to examine women who had no symptoms.[4] Traditional and religious taboos prevent examination of the female genitals by male doctors; the number of female doctors in Africa is very small, thus preventing adequate surveillance.

Control

After enumerating the enormous problems associated with surveillance of STD in Africa, one can hardly justify any talk on control programs for PID-associated agents. In short, therefore, one can summarize that control is minimal in certain countries and nonexistent in others.

Control policies should be largely or partly based on identification and adequate treatment of the pool of both symptomatic patients and asymptomatic gonococcal carriers. In Africa, only a few of the patients who suffer from gonorrhea are treated adequately in the few existing good centers; the much larger number of patients who report to various other practitioners receive inadequate therapy. Even though relatively resistant gonococci are becoming more common in East Africa,[9, 10] very few physicians are aware of effective drugs and adequate dosages for gonorrhea. Verhagen and associates[9] and Perine and colleagues[10] stress that the problem of resistant gonococci and antibiotic-resistant PID will continue to increase unless something is done to implement proper treatment and other control measures on a national level.

As already mentioned, contacts are hard to identify. Every effort to increase identification and location of contacts should be made. The efforts of both government and voluntary organizations must be stimulated for this purpose. These groups should increasingly recognize the seriousness of the morbidity and mortality associated with gonococcal PID. The African Union Against Venereal Diseases and Treponematoses (AUAVDT) recommended both points at its inaugural meeting in 1979. Increased education of all levels of medical personnel concerning STD also was recommended by AUAVDT. The same African Union has recommended increased health and sex education in schools. If these recommendations are implemented, a foundation will have been laid for avoiding STD and thus controlling PID.

Vaccination for gonococci, still in its infancy in the developed countries, cannot be suggested at the moment; even in the future a gonococcal vaccine is unlikely to benefit STD control programs in Africa. Thus, if an effective vaccine is found, it is likely to be so expensive that many African countries will not be able to afford it.

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Microbiologic techniques for the diagnosis of pelvic inflammatory disease in developing countries

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Pelvic inflammatory disease is a major medical problem that causes serious complications in a large number of the female population in many developing countries. It s estimated that between 20% and 30% of gynecologic admissions in these countries are due to PID. The alarmingly high prevalence of gonorrhea is probably a major cause of this syndrome. Diagnosis of PID on clinical grounds without laboratory verification is the rule. The various techniques for diagnosing PID are discussed briefly. Primarily because of very small health budgets the limitations faced by developing nations in the accurate diagnosis of PID are (1) a lack of adequately trained personnel, (2) inadequate clinic facilities, (3) low accuracy of clinical diagnosis, and (4) ill-equipped and understaffed laboratory services. Suggestions are offered for improvement of the present unsatisfactory situation. (AM. J. OBSTET. GYNECOL. 138:1091, 1980.)

PELVIC INFLAMMATORY DISEASE is a major medical problem in many developing countries. There are no accurate statistics to document the extent of the problem in all countries of tropical Africa, but studies in several medical centers indicate that PID and its sequelae are problems of considerable magnitude. In Uganda, between 22% and 30% of gynecologic admissions were for acute PID.[1] A similarly high proportion of gynecologic admissions in Nigeria are for PID.

The high incidence of PID in tropical Africa is due primarily to the appallingly high prevalence of gonorrhea, although other etiologic factors must be borne in mind, including agents that cause nongonococcal salpingitis, puerperal sepsis, and septic abortion. At one clinic in Uganda which served a population of 350,000, 18,000 cases of gonorrhea were recorded in 1971; in the same year, 56,000 cases of gonorrhea were encountered in England (population, 46 million).[2] Similarly, in various studies of nearly 1,000 women in Nigeria, gonorrhea was identified in 17% of those who complained of vaginal discharge and in 1% to 5% of other groups of asymptomatic women (Table I).[3, 4]

It has been suggested that 10% to 15% of women with cervical gonorrhea develop the complication of salpingitis.[5] Therefore, in tropical Africa, where the incidence of gonorrhea is very high, a large proportion of the female population will at one time or another suffer from PID. Unfortunately, in many women with PID, the condition goes unrecognized and untreated. This occurs because diagnostic facilities are inadequate and symptoms of PID, abdominal pain and fever, are ascribed to other conditions prevalent in the tropics, such as typhoid, dysentery, amebiasis, etc.

The acute and florid forms of PID are seen frequently in hospital outpatients and in casualty departments in developing countries. The subacute and mild forms either go unrecognized or are treated by nonmedical personnel, such as pharmacists, quacks, or native doctors; alternatively, the patient may attempt self-medication with antibiotics that are easily and readily obtainable. The antibiotic dose frequently depends on the amount of money the patient possesses. Women with the late sequelae of PID, infertility or reproductive problems, crowd the infertility clinics of many hospitals where they are discovered to have blocked fallopian tubes. Not only is gonorrhea one of the most common causes of involuntary infertility in tropical Africa, but it is also the cause of chronic pelvic pain and ectopic pregnancies.

The late sequelae of PID are well known, i.e., involuntary infertility, persistent pelvic pain, and increased frequency of ectopic pregnancy. In tropical Africa, the social consequences of PID add even greater misery to the health consequences of PID. Since procreation is of paramount importance in tropi-

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Table I. Incidence of sexually transmitted diseases among different groups of Nigerian women previously investigated (Osoba and Onifade, 1973, 1975, and 1979)

Groups of women	*No. investigated*	*% With gonorrhea*	*% With trichomoniasis*	*% With candidiasis*
Pregnant women	208	3.37	20.6	33.17
IUD acceptors	282	4.61	26.6	16.67
Women complaining of vaginal discharge	228	17.0	19.0	23.0
Asymptomatic women (illiterate women)	130	5.0	15.0	12.0
Women with infertility	151	1.32	20.5	26.6
Total	999			

Table II. Possible alternative approaches to gonococcal infection depending upon clinical laboratory resources*

	Women with		
Clinical laboratory resources	*Symptoms of cervicitis, urethritis, or endometritis*	*Symptoms of salpingitis*	*Asymptomatic contact*
Absent	Clinical diagnosis	Clinical diagnosis based on lower abdominal pain, abnormal uterine bleeding, recent vaginal discharge, or cervical motion tenderness	
Minimal	Clinical diagnosis plus Gram stain of endocervical-urethral discharge†	Clinical diagnosis plus Gram stain of endocervical-urethral secretion†	Gram stain of endocervical-urethral secretion†
Optimal (culture, identification, antimicrobial susceptibility)	Clinical diagnosis plus culture from endocervix and anal canal and/or urethra	Clinical diagnosis plus culture from endocervix and anal canal and/or urethra, or culture of culdocentesis or laparoscopy specimen	Culture from endocervix and anal canal and/or urethra

*Data from World Health Organization, 1978.

†Nonsensitive and nonspecific technique depending on clinical laboratory expertise.

Table III. Diagnostic practices of PID in developing countries

	Teaching or specialist hospital	*State or city hospital*	*District hospital*	*Health center, dispensary, aid post, etc.*
Personnel making diagnosis	Specialist or consultant	Any available physician	Nurse or medical auxiliary (rarely physician)	Nursing personnel, medical auxiliary, and nonmedical personnel*
Type of diagnosis	Clinical examination and bacteriologic	Mainly clinical, rarely bacteriologic	Clinical examination and/or history only	Clinical history only
Laboratory facilities	Good	Some (microscopy only)	None	None

*Many are treated by herbalists, native doctors, or quacks or they self-medicate.

cal Africa, infertile women may be divorced, abandoned, or replaced because they cannot fulfill their marital role.

I will now briefly describe the practices for diagnosing PID in developing countries and discuss the many limitations to accurate diagnosis.

Diagnosis of PID

Ideally, the diagnosis of PID should be a clinical one that is supported by culture of the endocervical and urethral secretions for gonococci, chlamydiae, anaerobes, and other pathogens; laparoscopic examination to confirm the clinical diagnosis; and culture of culdocentesis or laparoscopy specimens.

Alternative diagnostic approaches for gonococcal infections have been suggested depending upon available clinical laboratory facilities (Table II).[6] The ideal situation with optimal laboratory facilities exists in most hospitals in the industrialized nations where most patients suspected of having PID are seen. However, in the developing countries, PID in most patients is diagnosed and treated on clinical grounds or, at best, with use of Gram staining of the endocervical secretions; culturing facilities are rarely available.

Practices for diagnosing PID in developing countries. In developing nations, the diagnosis of PID varies from country to country and also within particular countries. The accuracy of the diagnosis depends

Table IV. Diagnosis of pelvic inflammatory disease

Type of diagnosis	*Developed countries*	*Developing countries*
Clinical history	Yes	Yes (mainly)
Clinical examination	Yes	Yes (mainly)
Laparoscopy and culdoscopy	Yes (commonly)	No (only few centers)
Laboratory		
A. Gonococcal		
1. Gram-stained smear	Yes	Yes (but only few centers)
2. Culture on selective media	Yes	Yes (but only few centers)
3. Sugar fermentation reaction	Yes	Rarely
4. Gonococcal complement fixation test	Yes	No
5. Antigonococcal pili antigen-binding assay	Yes (rarely)	No
6. Immunofluorescent staining	Yes	No
B. Nongonococcal		
1. Giemsa and iodine–stained smears	Yes	No
2. Papanicolaou-stained smears	Yes (rarely)	No
3. Specialized cultures, e.g., for *Chlamydia*, *Mycoplasma*, anaerobes, herpes virus, and cytomegalovirus	Yes (major centers)	No
4. Serologic tests, e.g., microimmunofluoresence, IgG chlamydia antibody	Yes	No
5. Serum protein changes, e.g., C-reactive protein, antichymotrypsin, and orosomucoid	Yes (major centers for research)	No

largely on the clinical acumen of the personnel who make the diagnosis and the distance from the facility to a main city. In most state capitals, the population is served by a specialist and/or a medical school. Here, gynecologists of consultant level will be found. The clinical diagnosis of PID will be backed by standard bacteriologic investigations, since the hospital will have a good laboratory (Table III). However, farther away from the main cities, diagnosis usually is made by paramedical personnel because of the shortage of medical personnel. The diagnosis is based on inexpert clinical examination and/or history only. No bacteriologic investigations can be pursued because laboratory facilities do not exist.

The majority of the population lives in rural areas in most countries and is served by health centers, dispensaries, and aid posts. These health facilities are often hundreds of miles away from the major cities and are linked to them by poor roads. Although most patients seek help in such centers, a good many turn to nonmedical practitioners, such as herbalists, native doctors, and quacks, or to self-medication with antibiotics obtained from the local market. Patients with the acute and florid forms of PID usually are referred from their local health facility to the nearest hospital. Although there is a physician at the hospital, the necessary trip over miles of very bad roads may worsen the patient's conditon or result in her death.

In short, the standard of diagnosis and management of PID in any developing country relates to the standard of the health care delivery system in that country.

Limitations of the diagnosis of PID in developing countries

The limitations of accurate diagnosis and adequate management of PID in developing countries cannot be divorced from the fact that these countries are nonindustrialized and have a limited health budget. This budgetary limitation makes impossible the provision of a good health care delivery system. The limitations in many developing countries are listed here.

Trained hospital personnel. Doctors, nurses, and laboratory technicians are in short supply in many developing countries, since the health budgets can allow for only one or two medical schools where these personnel can be trained. Consequently, great reliance is placed on an imported nonindigenous medical staff to man the larger hospitals; in the health centers in rural areas, paramedical staff, e.g., health assistants, medical auxiliaries, etc., are left to manage patients. The virtual absence of physicians in the districts and the rural areas decreases the accuracy of PID diagnosis, especially since laboratory facilities are lacking.

Clinical facilities. In the major cities and medical schools, clinic facilities are usually fair to good. Patients with PID who are seen in the outpatient departments are referred to gynecologists. However, in the district and rural hospitals, facilities usually are poor, and may be substandard. It is not uncommon to find that swabs for the collection of specimens, gloves, and specula are not available in such centers; consequently, a good clinical examination is not possible, no matter how enthusiastic the physician. Facilities for culdoscopy and

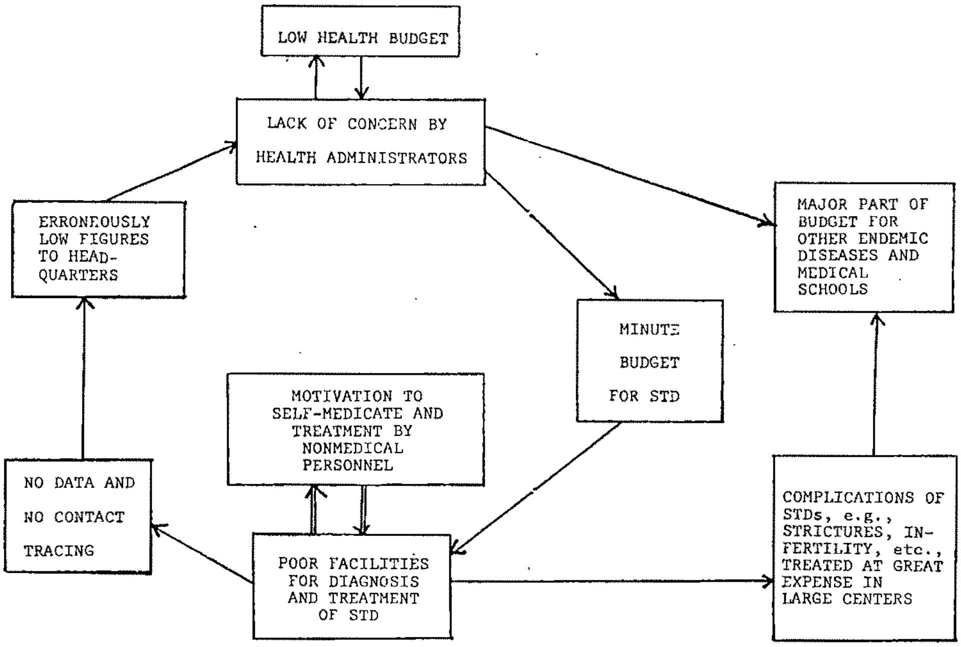
LOW HEALTH BUDGET
LACK OF CONCERN BY HEALTH ADMINISTRATORS
ERRONEOUSLY LOW FIGURES TO HEAD-QUARTERS
MAJOR PART OF BUDGET FOR OTHER ENDEMIC DISEASES AND MEDICAL SCHOOLS
MINUTE BUDGET FOR STD
MOTIVATION TO SELF-MEDICATE AND TREATMENT BY NONMEDICAL PERSONNEL
NO DATA AND NO CONTACT TRACING
POOR FACILITIES FOR DIAGNOSIS AND TREATMENT OF STD
COMPLICATIONS OF STDs, e.g., STRICTURES, INFERTILITY, etc., TREATED AT GREAT EXPENSE IN LARGE CENTERS

Fig. 1. The vicious circle of STD service in developing countries.

laparoscopy can be expected only in the medical schools and not in the service-oriented state hospitals or district hospitals.

Most countries have no organized services to deal with sexually transmitted disease (STD). At best, there may be a clinic in the state capital, managed by a general physician or paramedic. Such clinics manage or mismanage the mild and subacute forms of PID, whereas the most severe and acute forms are referred to the outpatient department.

Low accuracy of clinical diagnosis. It is well known that the clinical pattern of acute PID is, to a great extent, uncharacteristic. Even for the most competent clinician working in well established centers, the accuracy of clinical diagnosis ranges between 60% and 80%.[7–9] It is to be expected, therefore, that in the nonindustrialized nations, where most of the PID patients are seen by general physicians and paramedical staff, a lower degree of accuracy will be recorded.

The differential diagnosis of pelvic pain is difficult to make, and particularly so for subacute pain. In many tropical countries, several common endemic diseases (typhoid, dysentery, amebiasis, etc.) may be confused with PID, so that a diagnosis is still more difficult to make. In the hands of inexpert personnel, this confusion is even more severe. Other differential diagnostic problems include septic abortion, puerperal sepsis, and other etiologic agents which cause pelvic infections, such as tuberculosis, parasites, and fungi, all of which are endemic in many tropical countries. Furthermore, diagnostic difficulties are caused by self-medication by patients with subtherapeutic or inappropriate antibiotics, a practice widespread in many developing countries.

Laboratory services. Most diagnostic laboratories in developing countries are ill equipped, understaffed, and manned by improperly trained personnel. This stems invariably from inadequate budgetary allocations for general laboratory services. Most of these laboratories lack basic equipment and reagents to carry out bacteriologic isolation of genital pathogens, such as gonococci, chlamydiae, or mycoplasmas.

It is well known that the gonococcus is a fastidious organism, easily overgrown by intestinal microorganisms and vaginal flora. Thus, laboratories which use nonselective media (because of a lack of funds or for other reasons) will record a very low isolation rate for gonococci in cases of PID. The accuracy of such a laboratory is questioned further because, even in the best of hands and in well established centers, the frequency with which gonococci can be isolated from the cervix in women with PID varies from 2% to 80%.[8–11]

Many of the tests used in routine and research investigations of PID are available in most developed countries but not in the developing countries (Table IV). Performance of all these tests is laborious and expensive, and places a heavy burden on the laboratory. The burden is even greater in the developing countries.

At present in many nonindustrialized nations the diagnosis of PID on clinical grounds without pathologic support is the rule. I suggest that laboratories in the larger hospitals be encouraged to carry out microscopic and cultural diagnosis of gonococci as a minimum, including susceptibility testing of isolates. Where trained personnel are available, Giemsa-stained and iodine-stained smears may be performed to identify intracytoplasmic inclusions of chlamydiae.

Improvement of the present situation

Since tubal infection arises mainly as a complication of STDs, improvement in the diagnosis and management of PID must be related to the control of STD and the standard of venereal disease service in the particular country.

Willcox[12] accurately described the vicious circle which bedevils the developing countries, ensuring an appalling underdeveloped service to control STD, despite the high incidence of the disease and the common complications (Fig. 1). There tends to be lack of interest in the problems of STD by the health administrators, partly because they refuse to admit that there is an STD problem and partly because erroneously low figures are returned from the one or two clinics which serve the entire country. This results in the allocation of only a very small budget to deal with STD, whereas the major part of the budget is directed toward the more respectable communicable and endemic diseases and the medical schools. The overall effect is that facilities for diagnosis and treatment of STD and PID are poor. Therefore, patients are motivated to medicate themselves or to seek treatment from nonmedical personnel, such as pharmacists, herbalists, and native doctors, whereas the more privileged patients consult private practitioners. Consequently, the meager facilities are barely utilized and erroneously low figures are supplied to the health administrators, thus maintaining the vicious circle. Despite the neglect, considerable expense is associated with the complications of STD, such as strictures, infertility, ectopic pregnancies, etc.

In order to achieve an improvement in the present undesirable and unacceptable situation, the following suggestions are offered: (1) change attitudes of health administrators to recognize that serious STD and PID problems exist, (2) improve the budgetary allocations for health services in general and the control of STD in particular, (3) provide basic facilities for diagnosis and treatment of patients with STD, and the tracing of their contacts, (4) educate health authorities, medical personnel, and the public at large about STD, and (5) train a sufficient number of health personnel to operate a reasonably adequate STD control service.

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Problems and approaches to the control and surveillance of sexually transmitted agents associated with pelvic inflammatory disease in the United States

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The national gonorrhea control program has expanded since its inception in 1972 and now reflects new information and program evaluations. Initial program objectives to reduce the incidence of disease and the resistance of gonococci to antibiotics have been expanded to reduce the occurrence of pelvic inflammatory disease and to limit the sequelae of PID. The changes since 1972 in the national venereal disease control program have increased the number of people helping control programs and have enhanced our ability to control other sexually transmitted diseases. (AM. J. OBSTET. GYNECOL. 138:1096, 1980.)

THE COORDINATED national gonorrhea control effort began in late 1972, after a decade of annual increases in reported cases.[1] Compounding the problem of increasing cases, treatments had become progressively less effective as gonococci developed increasing resistance to antibiotics.[2] The program to control gonorrhea was built upon administrative and surveillance systems that had evolved during three decades of coordinated efforts by state and local health departments and the United States Public Health Service to control syphilis. In addition, many aspects of the control program were based on syphilis control concepts. Thus, screening, tracing of contacts, epidemiologic treatment, and effective treatment of cases were used to discover cases and limit disease transmission. Since this beginning, the gonorrhea control program has changed and reflects new information, experience, and program evaluations.

We review the evolution of the gonorrhea control effort, arbitrarily dividing the program into an initial phase, a middle phase which will be referred to as the "transmitter phase," and the current phase (Fig. 1). Finally, we suggest how changes influence our approaches to new problems; we use control of *Chlamydia trachomatis* as an example of a new problem.

Initial phase

The initial program objectives were to reduce disease incidence and the resistance of gonococci to antibiotics (Table I). To reduce disease incidence we sought to identify asymptomatic or unrecognized disease in women.[3] To reduce the resistance of gonococci to antibiotics we sought to minimize the use of ineffective treatments which favored the relatively resistant organisms. Thus, national gonorrhea treatment recommendations were made in 1972, and widespread use of them was promoted.

By 1975, all state and local health departments had implemented a gonorrhea screening program which obtained 9.0 million cultures per year. Broad participation was encouraged to identify the maximum number of infected women, to increase provider recognition of gonorrhea, and to identify the most important providers. Laboratory, logistic, and training support was organized to ensure that the culture screening efforts were effective.

Contact-tracing interviews with infected men were used to identify asymptomatic women; the interview–field investigation model used was that developed for syphilis. However, the large number of men with gonorrhea and the short incubation period of this infection led many local programs to explore alternative methods of referral of sexual partners.

At that time, public clinics were viewed as sites to treat men, to interview men, and to treat women discovered by the program. To accomplish these tasks, the primary change in many clinics was merely to increase

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clinic hours. Gram-stain smears replaced clinical diagnoses in men, primarily to help target sexual partner referral efforts on those men with gonococcal urethritis.

- Public education was used to create awareness of and support for the gonorrhea control program. Education for adolescents was designed to improve their recognition of and response to disease. As an integral part of the screening effort, medical personnel received information on recommended diagnostic and treatment methods.

The reporting of gonorrhea cases remained the primary surveillance mechanism, but many health departments also developed mechanisms to identify and follow important epidemiologic variables. Improved diagnostic practices, expanded case-finding activities, and increased reporting all improved disease surveillance. In addition, a multi-city surveillance system was established to measure trends in resistance of gonococci and relate these to treatment efficacy.[4]

Transmitter phase

During the second or transmitter phase, experience and theoretical consideration, which emphasized the greater importance of some disease transmitters, led to program changes.[5, 6] Efforts to identify patients in whom treatment failed and to limit the spread of their organisms received additional attention when penicillinase-producing *Neisseria gonorrhoeae* (PPNG) was recognized.[7]

In many areas, screening was targeted increasingly at patients who attended high-priority providers and who attended for tests of cure and repeat testing 6 to 12 weeks after treatment. Likewise, efforts to improve the effectiveness of screening led many programs to enhance quality control and speed reporting of results to providers and to ensure treatment of identified patients.

Patient counseling efforts began to change as many programs sought to implement tests of cure and rescreening. To improve compliance with these recommendations, the clinic staffs increasingly enlisted patients as essential partners[8] in disease control. Thus, the effectiveness of patients in referring their own sexual partners allowed programs to focus the traditional but more expensive interview–field investigation efforts on selected high-priority patients. Such patients included those in whom treatment failed, those who had positive rescreening tests, and other repeaters.

Clinic services received increasing attention during this period as the number of "important" people grew from a few patients with syphilis and their contacts, to men with gonorrhea, to all infected patients. In an effort to enhance compliance by first managing the patient's problems, many clinics began to expand services to encompass sexually transmitted diseases (STDs) in addition to gonorrhea and syphilis.[9] Likewise, efforts were made to improve staff sensitivity to patient needs and to modify clinic procedures. Increasingly, clinic facilities were reevaluated and their importance to the control efforts was emphasized.

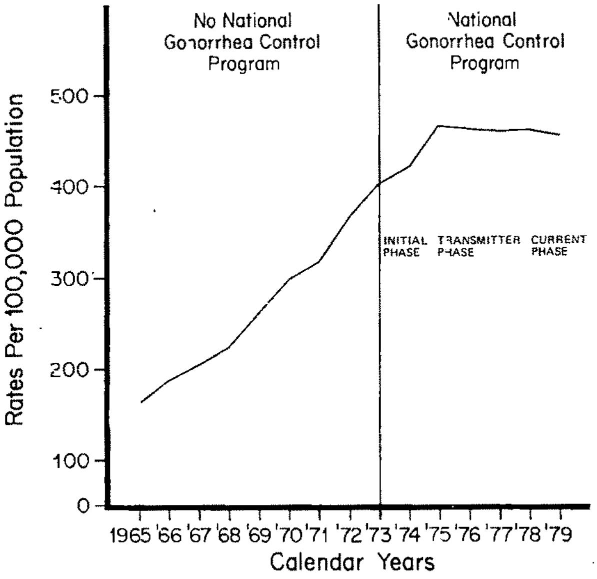

Fig. 1. Reported gonorrhea rates per 100,000 population in the United States for the calendar years 1965 through 1979.

With these changes in program focus, education efforts were directed increasingly at clinic patients and at high-risk groups. In addition, voluntary organizations were encouraged to undertake education efforts directed at the general public and to assist in reaching high-risk groups.

Collaboration between the laboratory and field staffs of state and local health departments allowed implementation of a PPNG surveillance system.[10] Detection of a case of PPNG led to intensive field investigations and follow-up, which limited spread of these organisms.

Current phase

In the current phase, program experiences, special studies, and analyses of national data clarified the public health importance of gonococcal pelvic inflammatory disease.[11–14] Therefore, program objectives were expanded to include limitation of the incidence and consequences of PID.

Although program activities had been associated with slightly declining gonorrhea rates since 1975 (Fig. 1), visits for PID to office-based practices had declined markedly since 1973[12] (Fig. 2). However, attention had

Table I. Evolution of national gonorrhea control program in the United States, 1972 to the present

	Initial phase	*Transmitter phase*	*Current phase*
1. Objectives	*Reduce incidence* *Reduce resistance*	Reduce incidence Reduce resistance *Limit PPNG*	Reduce incidence *Reduce PID* Reduce resistance Limit PPNG
2. Approaches	*Identify asymptomatic women* *Use effective therapy*	Identify asymptomatic women *Identify transmitters* Use effective therapy *Identify resistant organisms*	Identify *all infected* women *Recognize and manage PID* Identify transmitters *Identify untreated men* Use effective therapy Identify resistant organisms
3. Elements		(Focus of elements)	
A. Screening	*Screen women* *Quality cultures*	*Priority* screening *Test of cure* (TOC) *and rescreening* Quality cultures and *follow-up*	Priority screening *Hospital emergency room* TOC and rescreening of women Quality cultures and follow-up
B. Contact tracing	*Interview infected men*	*Counsel for infection* *Interview repeaters* Interview men	Counsel for infection *Interview patients with gonococcal PID* Interview repeaters Interview *others*
C. Clinic services	*Expand hours* *Perform smears*	*Manage genital problems* *Patient sensitivity* *Support control*	*Performance standards* *Evaluate for PID* *Collaborate with others*
D. Education	*General public* *Adolescents* *Providers*	*Infected clinic patients* *Repeaters* *High-risk groups* Providers General public	*Clinic staff* *All* clinic patients *Women with PID* Providers High-risk groups General public
E. Surveillance	*Reported cases* *Organism resistance*	Reported cases *PPNG* Organism resistance	Reported cases *PID in hospitals* PPNG Organism resistance

Table II. National gonorrhea control program, gonococcal PID initiative

1. Screen for gonorrhea in hospital outpatient departments
2. Identify "target hospitals"
3. Identify and interview women with gonococcal PID
4. Evaluate and treat sexual partners
5. Ensure apropriate management and follow-up for women with gonococcal PID

not been directed to secondary prevention, that is, the management of cases of PID. Studies conducted in hospital emergency rooms by several programs demonstrated the feasibility of identifying women with PID, ensuring appropriate management of such women, and identifying many previously untreated sexual partners.[15–17] Treatment of these men should lead to reduced transmission as well as a decrease in the risk of reinfection for the woman with PID.

Efforts to increase the effectiveness of the gonorrhea screening program continue; however, major emphasis is now placed on improved and expanded screening in hospital outpatient departments. As a result of screening in hospitals, interactions between health departments and hospitals are improving greatly.

Efforts toward counseling women who have gonococcal PID and referral of sexual partners are intended to educate the patients, ensure their adequate management and follow-up, and prevent reinfection by locating and treating sexual partners.

Standing orders for clinics which provide care that encompasses a variety of STDs have clarified the supervision and training needs of clinic personnel. The model STD prevention/training clinics are now a focus for improving the skills of clinic personnel and professionals during their formal education.[18]

Education efforts now focus on providers in clinics, emergency rooms, professional schools, and private practice. This training emphasizes improved management of individual cases and improved prevention efforts. Attempts to evaluate education activities aimed at patients are being extended to women with PID.

Surveillance activities now include the identification of women with PID in selected hospital emergency rooms. This surveillance system will allow local pro-

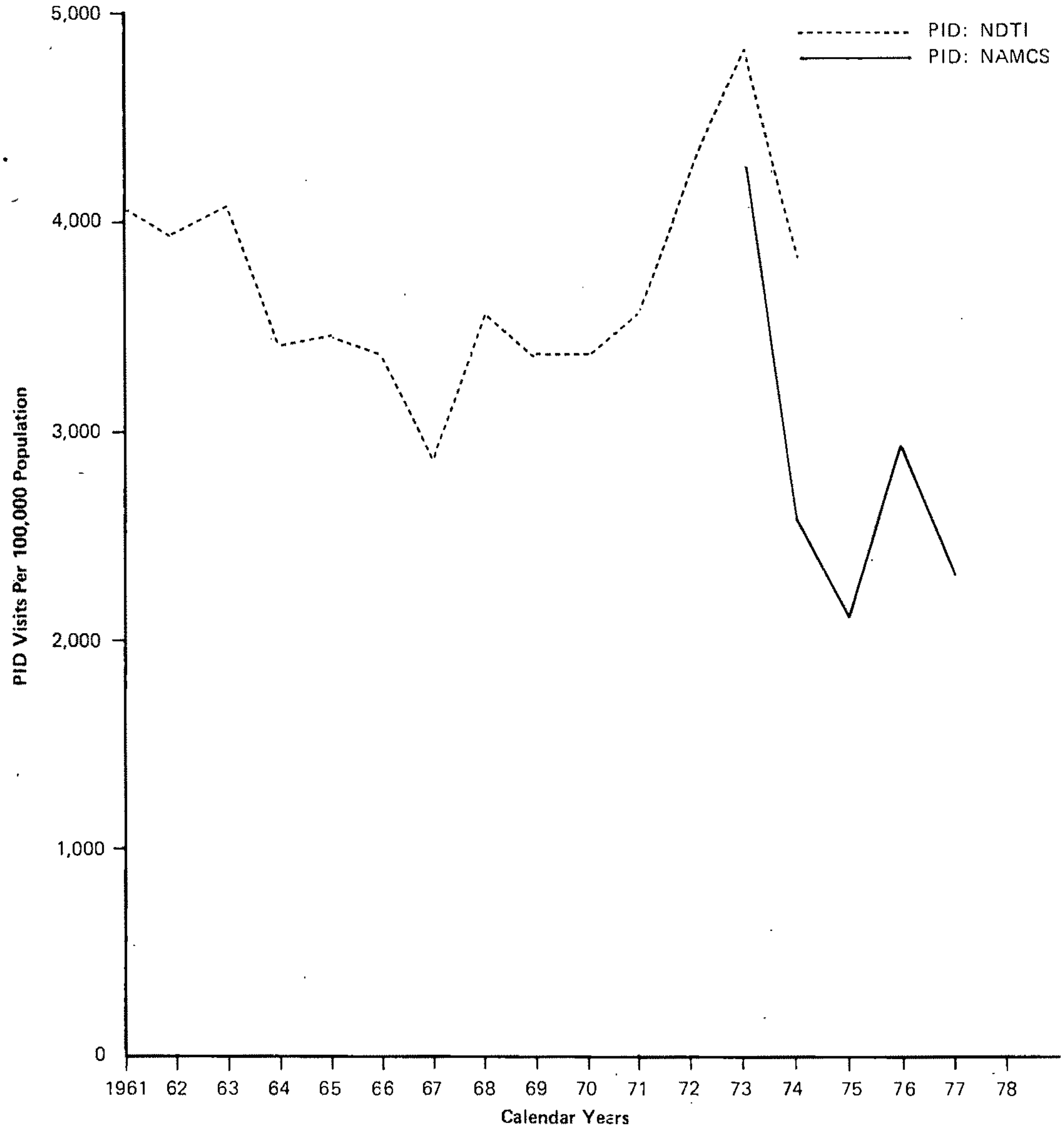

Fig. 2. Total PID visits among women. Visits to the offices of private physicians per 100,000 population in the United States for the calendar years 1961 through 1977. *NDTI,* National Disease and Therapeutic Index; *NAMCS,* National Ambulatory Medical Care Survey. Total PID is defined as salpingitis [acute, International Classification of Diseases, Amended (ICDA), No. 612; chronic, ICDA No. 613; and unqualified, ICDA No. 614, plus diseases of the parametrium and pelvic peritoneum, ICDA No. 616].

grams to evaluate PID trends and to adjust programs accordingly.

In summary, the gonococcal PID initiative is an effort to emphasize an aspect of gonorrhea control which has received inadequate attention (Table II). It is essential to develop surveillance and management systems for this problem. Collaboration with hospital emergency rooms must be improved. In most areas, collaboration will begin with improved gonorrhea screening which leads to the identification of cases, interviews, follow-up and referral of sexual partners.

Many aspects of the national veneral disease control program have changed since gonorrhea control began in 1972. These changes will enhance future efforts to control STDs. Thus, if the control of chlamydial infection were to begin, the objectives would include reductions in PID and perinatal syndromes as well as in disease incidence. The developing hospital surveillance systems would be adapted to measure these consequences and would supplement the current clinic surveillance systems for the nonreported cases of STD, including uncomplicated genital chlamydial disease. The tracing of contacts from hospital cases and symptomatic clinic cases would involve patients in follow-up and referrals of sexual partners as developed for gonococcal disease. Screening efforts would be focused on transmitters and on selected prenatal groups at high risk for disease. Improved clinic services will be able to attract the less symptomatic patients with chlamydial infection and to incorporate new procedures into their standing orders. The improved mechanisms for educating providers will facilitate changes in their performance. Finally, the many outside groups which support STD control efforts will ensure the best political climate for chlamydia control efforts.

In conclusion, we believe that the efforts to control venereal disease need diagnostic and treatment standards, an understanding of local disease epidemiology, and imaginative use of available resources. The process of change we have described depends on critical evaluations by a variety of people; without the participation of so many people, our national control efforts would be much less effective.

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Gonococcal pelvic inflammatory disease: Case-finding observations

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During a 20 month period, 110 women with gonococcal pelvic inflammatory disease and 165 women with uncomplicated gonorrhea were provided intensive case-finding services (interviewing of patients and tracing of contacts). Approximately three contacts per case were investigated, and 24.3% of the 859 male contacts were infected. Nearly two thirds (64.6%) of the infected contacts were asymptomatic. Active public health intervention was frequently necessary to persuade asymptomatic men to seek medical attention; removal of these men from the disease pool may serve to prevent reinfection of treated women and to diminish the transmission of gonorrhea. (AM. J. OBSTET. GYNECOL. 138:1101, 1980.)

ALTHOUGH GONORRHEA is reportable in the United States, its most prevalent complication, pelvic inflammatory disease, is not. Estimates suggest that 10% to 15% of gonococcal infections in women result in PID.[1] Public health efforts to control PID are hindered since most women with pain in the lower abdomen present in clinical settings in which case-finding services may not be readily available.[2] Thus, the epidemiologic characteristics of patients with gonococcal PID and the relationship of this condition to the overall transmission of gonorrhea are not well defined. Few case-finding reports have been published.

The present study was undertaken to determine the magnitude of the problem of gonococcal PID in Colorado Springs, Colorado, and to describe some of its epidemiologic features.

Material and methods

Between August, 1976, and March, 1978, we provided intensive case-finding services to 110 women with gonorrhea and acute salpingitis (the PID group) and 165 women with uncomplicated gonorrhea (the comparison group). These 275 individuals from whom *Neisseria gonorrhoeae* were isolated by modified Thayer-Martin culture were identified by eliciting the initial reason for presentation from attending physicians in Colorado Springs. Acute salpingitis was defined by a self-report of lower abdominal pain as a reason for seeking medical care, and was confirmed by the patient and her physician during subsequent interviews with the study staff. Women in the comparison group had gonorrhea, reported no pain in the lower abdomen to either attending physician or study staff, and were found to be infected through routine screening (51.3%) or presentation with symptoms other than pelvic pain (48.7%). None of the women in either group was a known or suspected gonorrhea contact; each presented in a variety of provider settings without any intervention on our part.

Case-finding consisted of three steps: (1) counseling about the natural history, complications, and significance of the disease, the need for test of cure, and the importance of the referral of contacts, (2) elicitation of contacts, and (3) when necessary, tracing of contacts. All traced contacts were offered epidemiologic treatment as recommended by the Center for Disease Control (CDC).[3]

Data on enrollees were recorded on medical charts, gonorrhea epidemiologic records (CDC 9.97), and ve-

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Table I. Utilization of medical facilities in Colorado Springs, Colorado, by women with gonorrhea

Facility	*Gonococcal PID*		*Comparison group*		*All others—no. reported*
	No. reported	*No. interviewed*	*No. reported*	*No. interviewed*	
Venereal disease clinic	35 (19.9%)	22 (20.0%)	74 (35.2%)	58 (35.1%)	509 (73.7%)
Emergency room	74 (42.0%)	46 (41.8%)	13 (6.2%)	10 (6.1%)	36 (5.2%)
Private physician	45 (25.6%)	28 (25.4%)	58 (27.6%)	46 (27.9%)	52 (7.5%)
Planned Parenthood	14 (8.0%)	9 (8.2%)	57 (27.2%)	45 (27.3%)	19 (2.7%)
Other	8 (4.5%)	5 (4.6%)	8 (3.8%)	6 (3.6%)	75 (10.9%)
Total	176 (100.0%)	110 (100.0%)	210 (100.0%)	165 (100.0%)	691 (100.0%)

Table II. Results of elicitation of contacts

Contacts named	*Gonococcal PID (N = 110)*	*Comparison group (N = 165)*	*Total (N = 275)*
Infected	86 (22.9%)	123 (25.4%)	209 (24.3%)
Asymptomatic*	51 (59.3%)	84 (68.3%)	135 (64.6%)
Not infected	151 (40.3%)	227 (46.9%)	378 (44.0%)
Not found	115 (30.7%)	122 (25.2%)	237 (27.6%)
Refused examination	23 (6.1%)	12 (2.5%)	35 (4.1%)
Total	375 (100.0%)	484 (100.0%)	859 (100.0%)

$\chi^2 = 11.854$; df = 3; $p < 0.01$

*Asymptomatic includes men who reported no symptoms or recalled one so minor (usually scant discharge without dysuria) that they would not have sought medical care without our intervention.

Table III. Ethnic distribution of cases and contacts

Ethnic group	*Gonococcal PID*			*Comparison group*		
		Contacts			*Contacts*	
	PID cases	*Infected*	*Total*	*Comparison cases*	*Infected*	*Total*
White	72 (65.5%)	32 (37.2%)	193 (51.5%)	134 (81.2%)	68 (55.3%)	312 (64.5%)
Black	19 (17.3%)	46 (53.5%)	130 (34.7%)	18 (10.9%)	45 (36.6%)	136 (28.1%)
Hispanic	17 (15.4%)	7 (8.1%)	41 (10.9%)	9 (5.5%)	9 (7.3%)	28 (5.8%)
Other	2 (1.8%)	1 (1.2%)	11 (2.9%)	4 (2.4%)	1 (0.8%)	8 (1.6%)
Total	110 (100.0%)	86 (100.0%)	375 (100.0%)	165 (100.0%)	123 (100.0%)	484 (100.0%)

nereal disease epidemiologic reports (CDC 9.2936), thus permitting description of each patient and her sexual partner by as many as 110 variables.

Results

Population selection. Of the 1,077 women with gonorrhea reported in Colorado Springs during the 20 month study period, 631 were contacts, 50 were repeaters, and seven had disseminated infections. These 688 women, plus three children, were excluded from the study. Of the 275 interviewed, 110 represented 62.5% of the 176 cases of gonococcal PID reported and 165 represented 78.6% of the 210 women reported with uncomplicated gonorrhea who were not contacts. Efforts were made to offer case-finding services to all women eligible for study; thus, two thirds of the patients with PID and four fifths of the comparison patients were reached. Most of these presented in settings other than public venereal disease clinics: Nearly half of the PID cases were reported by hospital emergency rooms, and most of the comparison cases came from types of clinical practice that screen for asymptomatic gonorrhea (Table I).

Patient characteristics. The patient selection process produced populations that were similar in age (two thirds in each group were between 17 and 23 years of age), occupational status (about 25% were unemployed; 8% were prostitutes), previous history of gonorrhea (about 25% had had a previous gonococcal infection), and previous history of PID (7% in each group). The comparison group had a higher proportion of married women (14.5% vs. 7.2% for the PID group) and a lower proportion of separated or divorced women (26% vs. 35% for the PID group). The

Table IV. Dispositions of infected contacts

	Gonococcal PID		Comparison group	
Infected contacts	*Asymptomatic*	*Total*	*Asymptomatic*	*Total*
Brought to treatment	37 (72.6%)	42 (48.8%)	70 (83.3%)	86 (69.9%)
Previously treated	14 (27.4%)	44 (51.2%)	14 (16.7%)	37 (30.1%)
Total	51 (100.0%)	86 (100.0%)	84 (100.0%)	123 (100.0%)

same proportion of women in each group used an intrauterine contraceptive device (10%) or used no contraception at all (40%). Significantly fewer women used oral contraceptives in the PID group (22.7%) than in the comparison group (43%) ($p < 0.01$).

Significant differences also were observed in racial distributions. The proportion of black and hispanic patients was twice as high in the PID group as in the comparison group (32.7% vs. 16.4%, respectively; $p < 0.01$). Race interacted with contraceptive use ($p < 0.01$); white women used oral contraceptives significantly more often than did black women. Thus, the lower proportion of white women (65.5%) in the PID group than in the comparison group (81.2%) may (at least, in part) have been due to differences in contraception.

Therapeutic management. Ninety percent of the women in the comparison group were treated with one of the regimens recommended for gonorrhea by the CDC. In contrast, 76% of the women with PID were so treated, and only 44% of this group initially received a course of therapy recommended for gonococcal salpingitis by the CDC.

Case-finding results and interview periods. The 275 women interviewed named 859 male contacts. On the average, three contacts per case were investigated ($\bar{X} = 3.1$, $\sigma = 2.9$, range = 1 to 22), and similar rates of infected contacts per case were found: 0.78 per case in the PID group and 0.75 per case in the comparison group. Overall, 24.3% (209 of 859) of the contacts were infected. Nearly 60% of the infected contacts in the PID group and 68% in the comparison group were asymptomatic (Table II). A higher proportion of PID contacts could not be examined (36.8% vs. 27.7% in the comparison group), including those who refused examination ($p < 0.01$). The PID group named more contacts per case (3.4) than the comparison group (2.9). This difference was not significant when time was standardized: Women in both groups named 1.3 different partners per month.

Flexible periods for interviewing contacts, adjusted to each patient's sociosexual history, led to additional contacts and the discovery of many new cases. About 35% of positive contacts in each group had their last sexual encounters with the patients more than 30 days before a diagnosis was made in the patients. The first three contacts named accounted for only 75% of the positive contacts. More than 15% of the infected contacts in the PID group and 13% in the comparison group were exposed to patients more than 90 days before culturing was performed in the patients.

Table V. Case-finding technique required for the 128 contacts newly brought to treatment

Case-finding technique	*Gonococcal PID*	*Comparison group*	*Total*
Self-referral	12 (28.6%)	39 (45.3%)	51 (39.8%)
Field follow-up	30 (71.4%)	47 (54.7%)	77 (60.2%)
Total	42 (100.0%)	86 (100.0%)	128 (100.0%)

$\chi^2 = 2.651$; df = 1; $p > 0.10$

Racial distribution. Differences were noted in the race of contacts by group and in the racial distribution of infected contacts. More than 80% of the women in the comparison group were white, but only 64.5% of their partners. Approximately 66% of the women in the PID group were white, but only 51.5% of their partners (Table III).

Distribution of infected contacts. In neither group were positive contacts evenly distributed. No infected contact was identified in 45% of the PID cases or in 45% of the comparison cases. Significant differences in distribution were observed in the 55% of cases in which at least one positive contact was identified. Twenty-four of the women in the PID group had 56 infected contacts. These 56 infections comprised nearly two thirds of all positive contacts in this group. For the comparison group, 24 of the women accounted for 54 (44% of positives) infected contacts. Thus, 21.8% of the women with PID and 14.8% of the comparison women (17.5% of all women) contributed over half of all infected contacts (110 of 209) in the study.

Disposition of infected contacts and self-referral behavior. Of the 209 total infected contacts, 128 were newly brought to treatment as a result of our investigations, and 81 were identified and treated before we intervened (Table IV). As might be expected, most (83.6%) of the cases newly brought to treatment were asymptomatic (107 of 128). Provided with equal opportunities to refer their own contacts to examination, members of the two groups behaved differently, although in neither group did self-referral seem to be a very effective case-finding tool. Of the 214 contacts to women in the PID group who were located and found eligible for examination, only 59 (27.6%) presented by this means; among the 234 contacts to women in the comparison group who were reached and had not been examined previously, 132 (40.7%) were self-referred ($p < 0.01$). Although the numbers are too small to be statistically significant ($p > 0.10$), only 12 (28.6%) of the 42 infected contacts newly brought to treatment in the PID group were self-referred, in contrast to 39 (45.3%) of 86 in the comparison group (Table V).

Discussion

The substantial number of infected, asymptomatic men in the sexual environments of the selected women is not surprising. Their presence was presumed, since detection of infection in each woman was an incidental event; each was self-referred to a physician or hospital primarily because of pelvic pain (PID group) or for routine gynecologic care (comparison group). To infer that some of their sexual partners might be asymptomatic was reasonable. In contrast, a smaller proportion of asymptomatic infections will be found in partners of women whose diagnoses were made as a result of contact tracing.[4, 5]

That the majority of these women present in clinical settings other than public venereal disease clinics emphasizes the importance of outreach efforts and of cooperation with the private medical sector on the part of control programs. Verification of diagnosis and treatment with the private physician should be accompanied by a request for permission to offer case-finding services to these selected gonorrhea patients. Women eligible for inclusion in the PID (176 cases) and comparison (210 cases) groups represented about one third of the 1,077 women reported in Colorado Springs during the study period. Epidemiologically, their importance stems from providing public health workers the opportunity to remove from the disease reservoir asymptomatic men who otherwise stand little chance of detection. Men are seldom screened for asymptomatic urethritis; even in public venereal disease clinics, yields from such a procedure are marginal.[6] Moreover, our findings suggest that self-referral of contacts by these women fails more often than it succeeds. Many of the asymptomatic men identified in our study were not easily motivated to seek medical attention; responsive health-seeking behavior was stimulated only by persistent appeals from our case-finders.

Persuading the women with PID to name their contacts was difficult; it was less so with comparison women. That the women in both groups generally held the belief that "men always know when they have gonorrhea" produced much of the resistance encountered. The requirement for relatively deep probing into the patient's sexual past (critical periods of more than 3 months were more common than shorter periods) presented additional difficulties. These obstacles were surmounted by the performance of quality contact interviews; few of these lasted less than an hour. Detailed explanations of the course of their infections (particularly for PID patients) and the use of visual aids seemed to be helpful, as did lending an empathetic ear to their medical and personal problems.

Conclusion

The data presented in the study suggest that gonorrhea case-finding services should be directed beyond the usual clinic populations, that these services should include the use of expanded interview periods, and that the active tracing of contacts should be implemented to assure the presentation of asymptomatically infected men. These efforts may serve to prevent infected men from reinfecting treated women and from transmitting gonorrhea to other sexually active but as yet uninfected women.

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Gonorrhea in the emergency department: Management, case follow-up, and contact tracing of cases in women

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From June, 1978, to June, 1979, all ten Columbus hospital emergency departments (EDs) participated in a program which provided test-of-cure, follow-up of untreated cases, and contact investigation of women infected with gonorrhea. Four hundred and eighty-nine women were found to be infected with gonorrhea; 99% of these were treated rapidly with recommended schedules. Sixteen percent of women with positive cultures required inpatient care for an average of 6.3 days. Seventy percent of women with positive cultures received counseling and test-of-cure for gonorrhea. Of 134 men treated as a result of these counseling efforts, 79 (59%) were asymptomatically infected. Source-spread analyses suggested that one third of asymptomatically infected male contacts had been infected for longer than 30 days. Only one fourth of women with gonococcal pelvic inflammatory disease (GC-PID) interviewed named male source contacts who were treated prior to her presentation with PID. Future approaches to GC-PID prevention must include cooperative efforts between health department clinics for sexually transmitted disease and hospital EDs. (AM. J. OBSTET. GYNECOL. 138:1105, 1980.)

Table I. Columbus Health Department—emergency department follow-up, hospitalization, and counseling, June, 1978, to June, 1979

	Culture-positive women	*No. hospital admissions*	*Test-of-cure and contact tracing*
PID	309	73 (23.6%) (Avg. = 6.3 days)	233 (75%)
Other	180	7 (3.9%) (Avg. = 5.9 days)	110 (61%)
Total	489	80 (16.4%) (Avg. = 6.25 days)	333 (68%)

WOMEN WITH GONOCOCCAL pelvic inflammatory disease (GC-PID) and other acute complications of gonorrhea often present to hospital emergency departments (EDs) for care.[1, 2] Management of these patients and their sexual partners is particularly difficult in these health care settings. During 1977, EDs participating in the Columbus (Ohio) gonorrhea screening program detected 648 gonococcal infections. Though 70% of 412 infected women were reported to be symptomatic, only 69% of these were treated at the time of their visit. Furthermore, only 25% of nonsymptomatic women were treated immediately. Follow-up of untreated patients was inconsistent despite efforts by hospital and health department personnel. Beginning in June, 1978, a surveillance system for GC-PID was established and all ten hospital EDs in Columbus participated with the Health Department in a program to manage ED patients with gonorrhea. This article describes the results of the program during the 12 month period from June, 1978, to June, 1979.

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Table II. Columbus Health Department—emergency department interviewing and contact tracing, June, 1978, to June, 1979

	No. interviewed	No. male contacts examined	Infections brought to treatment			
			Symptomatic	Asymtomatic	Total	Per interview
PID	233	280	39	62	101	0.43
Other	110	114	16	17	33	0.30
Total	333	394	55	79	134	0.40

Table III. Columbus Health Department—source of infection in patients with GC-PID

Presence of symptoms	Male sources	Men treated prior to PID episode	Referred for treatment
Yes	63	29	34
No	55	0	55
Total	118 (100%)	29 (25%)	89 (75%)

Methods

The Columbus Health Department manages a comprehensive gonorrhea control program centered in a modern clinic for sexually transmitted disease (STD) serving a metropolitan population of approximately 850,000. The clinic offers free STD services 5 days per week. All men and women attending the clinic with gonorrhea are interviewed and their contacts are offered free examination and treatment. The Health Department laboratory has supported culture screening for gonorrhea among 80,000 women per year since 1972. The ten Columbus hospitals participating in the program operate modern, well equipped, and adequately staffed EDs.

The gonorrhea management program included provision of a uniform culture system for *Neisseria gonorrhoeae,* telephone reporting of positive results, and rapid follow-up of untreated cases. For infected women, necessary treatment, follow-up test-of-cure, counseling, and referral of sex partners were centralized at the Health Department STD clinic. Each woman attending the clinic for test-of-cure was interviewed to elicit sexual contacts for the 90 days prior to her emergency visit. Infected men were classified as asymptomatic if they denied recent discharge or dysuria. To better understand the epidemiology of gonorrhea transmission among GC-PID patients in Columbus, we divided interviewed women into two groups: those with PID and others. Patients who had positive cultures were included in the PID group if their disease was diagnosed as PID by a physician, or if they presented with acute lower abdominal pain that responded to antibiotic therapy and the STD clinic physician felt PID was the probable diagnosis.

During this period of time careful histories and physical examinations, including pelvic examinations, were performed routinely on women attending the STD clinic. Additional cases of acute PID, mostly mild in severity, were diagnosed and treatments administered among this group of patients.

Results

Emergency department results. Four hundred and eighty-nine, or 8.9%, of the 5,472 ED cultures performed in women were positive for *N. gonorrhoeae.* Three hundred and nine of these were in patients with PID. Most of the other 180 women with gonorrhea sought care for nonspecific genitourinary symptoms. The mean age of these women, 20.8 years, was slightly younger than that of women with gonorrhea who attended the STD clinic. Most of the 263 men with positive cultures presented with signs and symptoms of urethritis and were treated immediately, but fewer than 60% of women were given adequate initial treatment. Through program efforts, the remaining men and all but seven of 489 infected women were treated within 1 week of their ED visit.

Table I lists the hospitalization and counseling totals. Six of the seven other women admitted from the ED were diagnosed as having disseminated gonococcal infection. No men with gonorrhea required inpatient care. Preadolescent girls and women who came to the ED with their sexual partners were less often included in the follow-up. This accounts for the difference in percent test-of-cure between PID and other groups.

Interviewing results. Results of interviewing and contact tracing from these infected women are shown in Table II. Seventy-nine (59%) of the 134 men who were infected but not yet treated had no symptoms. It was found that an additional 118 men had been treated for gonorrhea prior to the interview.

All infected men were also interviewed to determine sexual contacts. By comparing interview information from patients with GC-PID with that obtained from

Table IV. Columbus, Ohio—distribution of gonorrhea, June, 1978, to July, 1979

Provider	*No. men with GC*	*No. women with GC*	*No. women with GC-PID*
STD clinic	2,599 (70%)	1,376 (36.5%)	65 (17%)
Other providers (private and public)	1,101 (30%)	2,396 (63.5%)	313 (83%)
Total	3,700 (100%)	3,772 (100%)	378 (100%)

their infected contacts, we were able to identify the men who were probable sources of infection in 118, or 51%, of the 233 PID patients interviewed. Fifty-five (47%) of the 118 identified sources were asymptomatically infected and 63 (53%) had symptoms. Among symptomatic sources, only 17 (27%) had been infected for more than 7 days and only two for 1 month or longer. In contrast, 46 (84%) of the asymptomatically infected men had been infected for longer than 1 week with 19 (35%) infected longer than 1 month before treatment. As Table III indicates, only 25% of identified male sources had been treated prior to the woman's presentation with PID. None of the asymptomatically infected men independently sought treatment, while more than half of the symptomatic sources were referred for treatment after the PID episode.

PID surveillance. During this period of time in Columbus, 10% of the 3,772 reported gonorrhea cases in women were associated with a diagnosis of PID. This probably represents a considerable underestimate of the GC-PID rate since only four of 1,913 gonorrhea cases reported by private physicians, family planning clinics, and other providers were designated as PID. Sixty-five cases of GC-PID were diagnosed among 1,376 cases of gonorrhea seen in women in the STD clinic. These were generally mild, requiring referral infrequently and hospitalization rarely. Nearly two thirds of infected women seen in EDs (309 of 489) were diagnosed as having GC-PID. They represented more than 80% of reported cases of GC-PID. Table IV illustrates the distribution of the nearly 7,500 cases of gonorrhea reported during the period of study.

Discussion

Our findings confirm that women with serious pelvic infections associated with gonorrhea often seek care at hospital emergency departments.[1-4] Two thirds of the women with positive cultures in the EDs sought care for PID, while most others presented with symptoms suggestive of urethral syndrome, endometritis, or cervicitis/vaginitis.[2, 5-7] Nearly one quarter of patients with GC-PID required hospital admission and over 500 hospital bed-days were consumed by women first attending emergency departments with gonorrhea. The finding that no men were hospitalized underscores the sexual inequity of the burden of suffering from this infection.

We were able to identify infected probable sources in only 50% of cases of GC-PID. Since we required a verified laboratory diagnosis to designate a probable source, some failures could be attributed to transient or undetected infections in men. Additionally, some male sources of infection may have had contact prior to our arbitrary 90 day interviewing period, as suggested by Phillips and associates.[8] Our finding that nearly 50% of identified sources were asymptomatically infected with *N. gonorrhoeae* reinforces the importance of the asymptomatic man as a transmitter of infections that lead to complications.[4, 9, 10] Their potential for transmission and for reinfection of these women is high since more than one third of these asymptomatic men had been infected for longer than 1 month. When symptomatic men who fail to seek treatment are included, 75% of identified sources of GC-PID required referral for treatment.

Counseling of men attending the STD clinic with gonorrhea during the study period resulted in the identification of more than 640 infected women. Only 18 (2.8%) of these contacts presented to the Columbus EDs with gonococcal PID. Most other women treated for gonorrhea at the STD clinic are referred by their sex partners or they themselves suspect exposure to infection. Although 5% of women with gonorrhea attending the clinic were diagnosed as having PID, STD clinic cases were very mild and of short duration; such early detection and treatment probably are associated with a better fertility prognosis for these women.[11] These data support the logical presumption that rapid contact tracing and/or patient referral of contacts can prevent serious PID in these women.

Despite efficient intervention efforts, patients with gonococcal PID continue to present on a regular basis to Columbus EDs. Since only 25% of known male sources were treated prior to the woman's PID episode, more efficient contact tracing is not the whole solution. Further PID prevention efforts must include innovative approaches such as intensive, repeated screening of high-risk women and detection of asymptomatic male carriers through contact tracing from cases in women, and, perhaps, screening of groups of high-risk men.

In communities where STD clinics serve a large proportion of men infected with gonorrhea and where hospital EDs care for a large portion of women with GC-PID, cooperative efforts are crucial for the control of gonorrhea and prevention and management of GC-PID.

We gratefully acknowledge the assistance of the staffs of the Columbus Health Department laboratory and STD clinic and the emergency department staffs of Columbus hospitals.

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A problem-oriented gynecology clinic for patients with sexually transmitted diseases

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A problem-oriented clinic designed to provide continuity of care for female patients with sexually transmitted diseases (STDs) is described. The primary goal of the clinic is to ensure adequate follow-up care for individuals diagnosed as having pelvic inflammatory disease. Functions of the clinic also include follow-up care for patients with positive gonorrhea cultures and consultation for individuals with other STDs. From the time of initiation of the clinic in January, 1976, until November 15, 1979, 4,305 patients were seen. A stratified random sample of 190 of these patients revealed the reasons for attending the clinic to be: (1) follow-up for PID, 85 patients (44.7%), (2) positive gonorrhea culture, 63 (36.3%), (3) unsatisfactory gonorrhea culture, 14 (7.4%), (4) gonorrhea contact, 10 (5.3%), and (5) other, 12 (6.3%). Complete follow-up has been obtained on 3,935 (91.4%) of the patients. Of the 370 patients who did not return for follow-up visits, 336 were reported to the local health department for retrieval. There were 34 patients in whom the follow-up status could not be determined. The establishment, operation, advantages, and disadvantages of the clinic are discussed. (AM. J. OBSTET. GYNECOL. 138:1109, 1980.)

PELVIC INFLAMMATORY DISEASE is a frequently occurring complication of gonococcal infections.[1] It occurs in young, sexually active women and is often difficult to differentiate from other illnesses.[2] PID is associated with significant sequelae regarding fertility[3] and it can be confused with life-threatening problems such as acute appendicitis and ectopic pregnancy. The difficulties in establishing firm diagnostic criteria for PID have resulted in it remaining a diagnosis of exclusion, in which case the patient with this diagnosis must be followed carefully to preclude serious medical problems.

Most patients diagnosed as having PID are seen in outpatient clinics and emergency rooms.[4] Follow-up evaluations for patients seen in this setting are usually poorly structured and are frequently discouraged. When follow-up evaluation does occur it is rarely performed by the same clinician who originally treated the patient. Therefore, a lack of continuity in care usually results when there are no protocols to standardize patient management.

Because of the difficulties encountered in the management of patients with PID, we established a clinic to provide follow-up care for these individuals. An additional objective of the clinic was to standardize care for female patients with other sexually transmitted diseases (STDs). Our experiences in the establishment and management of this clinic are the subject of this report.

Personnel, materials, and methods

Personnel.

Patient population. Initially the clinic was established to provide follow-up care for patients seen in the emergency room and diagnosed as having PID. We progressively expanded the criteria for clinic evaluation to include: (1) female patients with positive gonorrhea cultures obtained at all in-hospital clinic sites, (2) individuals in whom a gonorrhea culture was unsatisfactory for diagnosis, (3) patients having sexual contact with individuals known to have gonorrhea, (4) individuals with other sexually transmissible diseases, such as trichomoniasis, condyloma accuminata, herpes simplex virus infections, syphilis, and nonspecific vag-

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initis, (5) patients referred from inpatient and outpatient facilities to rule out STDs, and (6) follow-up for patients recently hospitalized for pelvic infections.

Administrative staff. The clinic has one full-time secretary who coordinates clinic appointments, reviews and posts laboratory reports, maintains records of patient visits, and completes morbidity reports. This person also functions as a liaison with the local health department to ensure that appropriate care is provided for the sexual partners of the clinic attendees. Coordination of the clinic's activities with other facilities in the hospital is done also.

Clinical staff. The primary health care provider in the clinic is a physician extender, either a physician's assistant or nurse practitioner. Gynecologic house officers also assist in providing patient care. Attending physicians, although not always present in the clinic, are available for consultation. Family practice house officers, emergency medicine house officers, nurse practitioner students, physician's assistant students, and medical students also assist periodically, under close supervision, in the care of the clinic patients. Consultation in all speciality areas is available on an immediate basis.

Nursing staff. A full-time registered nurse in charge of the gynecology clinic supervises the nursing aspects of the pelvic infections clinic. This individual ensures that adequate nursing assistant personnel are available to meet the clinic needs. Additionally, nurses administer prescribed therapy and provide some patient education.

Materials.

Physical facilities. An appropriate number of examining rooms in the gynecology clinic are set aside for conducting the clinic at specified intervals in order to meet the needs of the clinicians and the anticipated patient attendance. These rooms are stocked appropriately with diagnostic aids to meet the needs of this clinic.

Medical record. A chart designed for problem-oriented care of patients attending an STD clinic is employed. Historical data include: (1) age, (2) parity, (3) menstrual history, (4) contraceptive choice, and (5) reason for attending the clinic. The physical examination evaluates: (1) vital signs, (2) major body sites involved by STDs, and (3) a checklist for pelvic examination findings.

Data card. A 5 by 8 inch summary data card is maintained on each patient. This is attached to the front of the chart and indicates the dates, diagnosis, laboratory results, and therapies for each visit. This is employed to decrease the bulkiness of the medical record and facilitates evaluation of patients.

Data book. A log book of patient visits is kept. This volume contains: (1) the clinic date, (2) the patient's name, (3) an identifying history number, and (4) the reason for attending the clinic. The clinic attendance is summarized in tabular form at the completion of each day, indicating the number of patients scheduled, the number that attended, and what type of care was provided.

Methods. All patients referred to the clinic are seen and evaluated, irrespective of their problem. Problems not related to gynecologic infectious diseases are referred to appropriate agencies.

The primary thrust is to provide follow-up for patients diagnosed in the emergency room as having pelvic inflammatory disease. Although all patients for whom this diagnosis is entertained are evaluated, we have established guidelines for the diagnosis. These include the patient having at least four of seven criteria which include: (1) abdominal tenderness, (2) abdominal rebound tenderness, (3) cervical displacement tenderness, (4) adnexal tenderness, (5) adnexal mass, (6) white blood cell count in excess of $10,000/mm^3$, and (7) oral temperature higher than 38° C. Once the diagnosis is made and other diagnoses have been excluded, therapy is initiated by protocol. Therapy includes either procaine penicillin G (4.8 megaunits intramuscularly) plus probenecid (Benemid, 1 gm, by mouth stat) and then ampicillin (500 mg by mouth four times daily for 10 days) or tetracycline (1.5 gm stat followed by 500 mg by mouth four times daily for 10 days). Each patient is instructed to return to the pelvic infections clinic within 24 to 72 hours irrespective of how she feels, unless her condition worsens. All patients are instructed as to the signs and symptoms of ruptured ectopic pregnancy and progressive peritonitis.

Upon arrival at the clinic the patient is evaluated by the clinic staff. A problem-oriented record is completed on each patient. Physical findings at the time of the visit are recorded and compared to previous clinic or emergency room records when appropriate. The patient is counseled by the clinician regarding the nature of the problem, with a reemphasis by the nurse. Patients receiving therapy are instructed to return within 4 to 7 days of completion of treatment for test-of-cure evaluation. Patients with other clinical entities are managed accordingly. Those individuals returning for test-of-cure evaluations are requested to return for rescreening 6 weeks later.

Results

Upon initiation the clinic was operated on a daily basis and required minimal time and personnel. Initially the daily load varied between one and three patients. Daily visits to the emergency room were needed to reinforce the concept of the clinic. With time more patients were referred, and currently 20 to 30 patients are scheduled for each clinic session that meets for three ½ day sessions per week. Approximately 50% of the scheduled patients fail to show up on a given day. The majority of patients who fail to return are those who have been scheduled for rescreening or repeat cultures. The percentage of kept follow-up visits for patients with PID is 75% for immediate evaluation and 65% for test-of-cure. When patients fail to return as scheduled, efforts are made to contact and reappoint them. We attempt to reschedule them at their convenience, when feasible. This has resulted in excellent follow-up.

During the period between January 1, 1976, and November 15, 1979, 4,305 patients were scheduled to be seen and 10,376 visits were made to the clinic. There was an average of 2.4 visits per patient. A stratified random sample of 190 patients was obtained. Reasons for attending the clinic among patients in this sample were: (1) follow-up visits for PID, 85 patients (44.7%); (2) patients with positive gonorrhea cultures returning for therapy, 69 (36.3%); (3) patients with unsatisfactory gonorrhea cultures returning for repeat cultures, 14 (7.4%); (4) patients having a history of sexual contact with individuals diagnosed as having gonorrhea, 10 (5.3%); and (5) a miscellaneous group of individuals seen for vaginitis, follow-up of recent hospitalization, and rescreening, 12 (6.3%). Follow-up evaluations of 3,935 (91.4%) of the 4,305 patients were obtained. There were 370 patients who failed to return for the visits as requested. The local health department was asked to assist in providing follow-up of 336 of these 370 patients. There was inadequate information on 34 patients and their follow-up status could not be determined.

The clinic has been employed successfully for training purposes. Gynecology, emergency medicine, and family practice house officers periodically rotate through the clinic. This facility in conjunction with a local health department clinic, staffed by the same clinicians, provides training in the management of STDs for medical students, house officers, and physician extenders. The clinic is also used for conducting clinical update courses in the management of patients with STDs.

Discussion

The need to establish a clinic to provide problem-oriented care for female patients with STDs can be questioned in some settings. The decision should be made on the basis of population served and the availability of alternate facilities. The establishment of such clinics, however, offers many advantages and very few disadvantages.

The clinical diagnosis of PID has been determined to be in error as frequently as 35% of the time.[2] Conditions with clinical presentation similar to that of PID include: endometriosis, ovarian cysts, intrauterine pregnancy, ectopic pregnancy, acute appendicitis, and other gastrointestinal problems. Both ectopic pregnancy and acute appendicitis, if not managed aggressively, are life-threatening illnesses. Although we have no firm statistics regarding this phenomenon after 4 years of operation, the senior author recalls eight cases of ectopic gestation and two cases of acute appendicitis misdiagnosed initially as PID that were retrieved from the problem-oriented clinic. Whether these patients would have been treated similarly if this clinic had not been available certainly cannot be determined.

We have been very liberal with our indications for diagnostic laparoscopy in patients attending this clinic. We have employed this technique in all patients who have not responded promptly to therapy and in those in whom the diagnosis was questionable. Because of laparoscopic examinations we recognize that endometriosis and ovarian cysts are frequently misdiagnosed as PID. We feel that the clinic has allowed us to focus our attention on a group of patients who are frequently misdirected from clinic to clinic and in whom no substantiated diagnosis is made.

Currently there are many antibiotics available for treatment of pelvic infections. The wide array of agents can result in many different forms of therapy being employed, some of questionable effectiveness. The establishment of the clinic has allowed us to initiate therapy protocols in order to evaluate various drugs. The establishment of standard regimens with notations of their effectiveness can be used as a data base to systematically study other antibiotics.

Training in the management of patients with STDs is not widely available in the United States. Additionally, this usually is not part of a medical school curriculum. The establishment of the problem-oriented clinic and its coordination with health department facilities have allowed us to initiate such a training program. We have used the facility to train house officers in various specialties, physician extenders, and medical students. Additionally, we have employed the clinic to conduct physician update training programs in the management of the female patient with STD. We think that the facility has been invaluable for this purpose.

The clinic is also employed as a referral agency for all gonorrhea cultures obtained in the hospital. Positive results are followed up appropriately by the clinic secretary. This ensures either that patients will be brought to therapy at the hospital or that local health department personnel will be made aware of the patients. This also decreases duplication of efforts in retrieving patients.

The major disadvantage of this clinic is that only one member of the couple with STD is evaluated. It would be more ideal to conduct the clinic in a setting where both parties could be evaluated. There is the advantage, however, of the same staff operating the local health department clinic; therefore continuity of care, although not in the same physical location, can be accomplished.

In conclusion, we feel that we have fulfilled the initial goal of the clinic in providing high-quality, standardized, follow-up care for patients diagnosed as having PID. Additionally, we feel that we have been able to provide many other services, such as a referral center and teaching facility, that otherwise would not have been accomplished.

The authors would like to express their sincere appreciation to the gynecology staff at The Johns Hopkins Hospital. Without their help, this clinic would not be a reality.

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December 15, 1980
volume 138, number 8
Index number

American Journal of OBSTETRICS AND GYNECOLOGY

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Description: The Cu-7 has coiled around the vertical limb 89 mg. of copper wire providing approximately 200 mm² of exposed copper surface area. The Tatum-T has coiled around the vertical limb 120 mg. of copper wire providing approximately 210 mm² of exposed copper surface area.

Contraindications: Not to be inserted when any of the following conditions exist: pregnancy or suspicion of pregnancy; abnormalities of the uterus resulting in distortion of the uterine cavity; acute pelvic inflammatory disease or history of repeated pelvic inflammatory disease; postpartum endometritis or infected abortion in the past three months; known or suspected uterine or cervical disease such as hyperplasia or carcinoma including unresolved, abnormal "Pap" smear; vaginal bleeding of unknown etiology; untreated acute cervicitis until infection is controlled; diagnosed Wilson's disease; known or suspected allergy to copper; previous ectopic pregnancy; significant anemia; and valvular heart disease, leukemia, or use of chronic corticosteroid therapy.

Warnings

1. Pregnancy: (a) Long-term effects. The long-term effects on the offspring of the presence of copper in the uterus when pregnancy occurs are unknown.

(b) Septic abortion. Reports have indicated an increased incidence of septic abortion associated in some instances with septicemia, septic shock and death in patients becoming pregnant with an IUD in place. Most of these reports have been associated with the mid-trimester of pregnancy. In some cases, the initial symptoms have been insidious and not easily recognized. If pregnancy should occur with a Cu-7 or Tatum-T in situ, the IUD should be removed if the thread is visible or, if removal proves to be or would be difficult, interruption of the pregnancy should be considered and offered to the patient as an option, bearing in mind that the risks associated with an elective abortion increase with gestational age.

(c) Continuation of pregnancy. If the patient chooses to continue the pregnancy and the Cu-7 or Tatum-T remains in situ, she must be warned of increased risk of spontaneous abortion and increased risk of sepsis, including death. The patient must be closely observed and advised to report immediately all abnormal symptoms, such as flu-like syndrome, fever, abdominal cramping and pain, bleeding, or vaginal discharge, because generalized symptoms of septicemia may be insidious.

2. Ectopic pregnancy: (a) Pregnancy which occurs with an IUD in situ is more likely to be ectopic than pregnancy occurring without an IUD. Therefore, patients who become pregnant while using a Cu-7 or Tatum-T should be carefully evaluated for the possibility of ectopic pregnancy.

(b) Special attention should be directed to patients with delayed menses, slight metrorrhagia and/or unilateral pelvic pain, and to those patients who wish to interrupt a pregnancy occurring in the presence of a Cu-7 or Tatum-T, to determine whether ectopic pregnancy has occurred.

3. Pelvic infection: An increased risk of pelvic inflammatory disease associated with the use of IUDs has been reported. While unconfirmed, this risk appears to be greatest for young women who are nulliparous and/or who have multiple sexual partners. Salpingitis can result in tubal damage and occlusion, thereby threatening future fertility. Therefore, it is recommended that patients be taught to look for symptoms of pelvic inflammatory disease. The decision to use an IUD in a particular case must be made by the physician and patient with consideration of a possible deleterious effect on future fertility.

Pelvic infection may occur with a Cu-7 or Tatum-T in situ and at times result in the development of tubo-ovarian abscesses or general peritonitis. The symptoms of pelvic infection include: new development of menstrual disorders (prolonged or heavy bleeding), abnormal vaginal discharge, abdominal or pelvic pain, dyspareunia, fever. The symptoms are especially significant if they occur following the first few cycles after insertion. Appropriate aerobic and anaerobic bacteriologic studies should be done and antibiotic therapy initiated promptly. If the infection does not show marked clinical improvement within 24 to 48 hours, the Cu-7 or Tatum-T should be removed and continuing treatment reassessed on the basis of results of culture and sensitivity tests.

4. Embedment: Partial penetration or lodging of the Cu-7 or Tatum-T in the endometrium or myometrium can result in difficult removal. This may occur more frequently in smaller uteri. *See removal instructions.*

5. Perforation: Partial or total perforation of the uterine wall or cervix may occur, usually during insertions into patients sooner than two months after abortion or delivery, or in uterine cavities too small. The possibility of perforation must be kept in mind during insertion and at the time of any subsequent examination. If perforation occurs, laparotomy or laparoscopy should be performed as soon as medically feasible and the Cu-7 or Tatum-T removed. Abdominal adhesions, intestinal penetration, intestinal obstruction, and local inflammatory reaction with abscess formation and erosion of adjacent viscera may result if the Cu-7 or Tatum-T is left in the peritoneal cavity.

6. Medical diathermy: The use of medical diathermy (short-wave and microwave) in a patient with a metal-containing IUD may cause heat injury to surrounding tissue. Therefore, medical diathermy to the abdominal and sacral areas should not be used on patients using a Cu-7 or Tatum-T.

7. Effects of copper: Additional amounts of copper available to the body from the Cu-7 or Tatum-T may precipitate symptoms in women with undiagnosed Wilson's disease. The incidence of Wilson's disease is 1 in 200,000.

Precautions

1. Patient counseling. Prior to insertion the physician, nurse, or other trained health professional must provide the patient with the Patient Brochure. The patient should be given the opportunity to read the brochure and discuss fully any questions she may have concerning the Cu-7 or Tatum-T as well as other methods of contraception.

2. Patient evaluation and clinical considerations. (a) A complete medical history should be obtained to determine conditions that might influence the selection of an IUD. A physical examination should include a pelvic examination, "Pap" smear, gonorrhea culture and, if indicated, appropriate tests for other forms of venereal disease. The physician should determine that the patient is not pregnant.

(b) The uterus should be carefully sounded prior to insertion to determine the degree of patency of the endocervical canal and internal os, and the direction and depth of the uterine cavity. Exercise care to avoid perforation with the sound. DO NOT USE THE INSERTION INSTRUMENT AS A SOUND. In occasional cases, severe cervical stenosis may be encountered. Do not use excessive force to overcome this resistance.

(c) The uterus usually sounds to a depth of 6 to 8 cm. Insertion into a uterine cavity measuring less than 6.5 cm. by sounding may increase the incidence of pain, bleeding, partial or complete expulsion, perforation, and possibly pregnancy.

(d) To reduce the possibility of insertion in the presence of existing undetermined pregnancy, the optimal time for insertion is the latter part of the menstrual flow or one or two days thereafter. The Cu-7 or Tatum-T should not be inserted post partum or post abortion until involution of the uterus is complete. The incidence of perforation and expulsion is greater if involution is not complete.

It is, however, necessary to place the Cu-7 or Tatum-T as high as possible within the uterine cavity to help avoid partial or complete expulsion that could result in pregnancy.

Physicians are cautioned that it is imperative for them to become thoroughly familiar with the instructions for use before attempting placement of the Cu-7 or Tatum-T.

(e) IUDs should be used with caution in those patients who have anemia or a history of menorrhagia or hypermenorrhea. Patients experiencing menorrhagia and/or metrorrhagia following IUD insertion may be at risk for the development of hypochromic microcytic anemia. Also, IUDs should be used with caution in patients receiving anticoagulants or having a coagulopathy.

(f) Syncope, bradycardia or other neurovascular episodes may occur during insertion or removal of IUDs, especially in patients with a previous disposition to these conditions.

(g) Patients with valvular or congenital heart disease are more prone to develop subacute bacterial endocarditis than patients who do not have such disease. Use of an IUD in these patients may represent a potential source of septic emboli.

(h) Use of an IUD in patients with acute cervicitis should be postponed until proper treatment has cleared up the infection.

(i) Since the Cu-7 or Tatum-T may be partially or completely expelled, patients should be reexamined and evaluated shortly after the first postinsertion menses, but definitely within three months after insertion. Thereafter annual examination with appropriate medical and laboratory evaluation should be carried out. The Cu-7 or Tatum-T should be replaced every three years.

(j) The patient should be told that some bleeding or cramping may occur during the first few weeks after insertion, but if these symptoms continue or are severe she should report them to her physician. She should be instructed on how to check after each menstrual period to make certain that the thread still protrudes from the cervix and cautioned that there is no contraceptive protection if the Cu-7 or Tatum-T has been expelled. She should also be cautioned not to dislodge the Cu-7 or Tatum-T by pulling on the thread. If a partial expulsion occurs, removal is indicated and a new Cu-7 or Tatum-T may be inserted. The patient should be told to return within three years for removal of the Cu-7 or Tatum-T and for replacement if desired.

(k) A copper-induced urticarial allergic skin reaction may develop in women using a copper-containing IUD. If symptoms of such an allergic response occur, the patient should be instructed to tell the consulting physician that a copper-containing device is being used.

(l) The Cu-7 or Tatum-T should be removed for the following medical reasons: menorrhagia and/or metrorrhagia producing significant anemia; uncontrolled pelvic infection; intractable pain often aggravated by intercourse, dyspareunia; pregnancy, if the thread is visible; endometrial or cervical malignancy; uterine or cervical perforation; or any indication of partial expulsion.

(m) If the retrieval thread cannot be seen it may have retracted into the uterus or have been broken off, or the Cu-7 or Tatum-T may have been expelled. Localization usually can be made by feeling with a probe; if not, x-ray or sonography can be used. When the physician elects to recover a Cu-7 or Tatum-T with the thread not visible, the removal instructions should be considered.

(n) If any patient with a Cu-7 or Tatum-T suddenly develops overt clinical hepatitis or abnormal liver function tests, appropriate diagnostic procedures should be initiated.

Adverse Reactions: Perforations of uterus and cervix have occurred. Perforation into the abdomen has been followed by abdominal adhesions, intestinal penetration, intestinal obstruction, local inflammatory reaction with abscess formation and erosion of adjacent viscera. Pregnancy has occurred with the Cu-7 or Tatum-T in situ and when either has been partially or completely expelled.

The incidence of spontaneous abortion, when conception occurs with intrauterine devices in situ, appears to be increased over that in unprotected women. Insertion cramping, usually of no more than a few seconds' duration, may occur; however, some women may experience residual cramping for several hours or even days. Intermenstrual spotting or bleeding or prolonged or increased menstrual flow may occur.

Pelvic infection including salpingitis with tubal damage or occlusion has been reported. This may result in future infertility. Complete or partial expulsion of the Cu-7 or Tatum-T may sometimes occur, particularly in those patients with uteri measuring less than 6.5 cm. by sounding. Urticarial allergic skin reaction may occur. The following complaints have also been reported with IUDs although their relation to the Cu-7 or Tatum-T has not been established: amenorrhea or delayed menses, backaches, cervical erosion, cystic masses in the pelvis, vaginitis, leg pain or soreness, weight loss or gain, nervousness, dyspareunia, cystitis, endometritis, septic abortion, septicemia, leukorrhea, ectopic pregnancy, difficult removal, uterine embedment, anemia, pain, neurovascular episodes including bradycardia and syncope secondary to insertion, dysmenorrhea, and fragmentation of the IUD.

Clinical Efficacy: In clinical trials, use effectiveness was determined as follows for parous and nulliparous women, as tabulated by the life table method. (Rates are expressed as cumulative events per 100 women through 12, 24, and 36 months of use.)

Cu-7 experience encompasses 387,131 woman-months of use, including 12 months for 11,517 women, 24 months for 7 895, and 36 months for 3,661.

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Cumulative pregnancy rates were:

	12 Months		**24 Months**		**36 Months**	
	Parous	**Nulliparous**	**Parous**	**Nulliparous**	**Parous**	**Nulliparous**
Cu-7	1.9	1.7	2.9	2.6	3.4	3.4
Tatum-T	3.0	2.1	4.9	4.5	6.0	5.8

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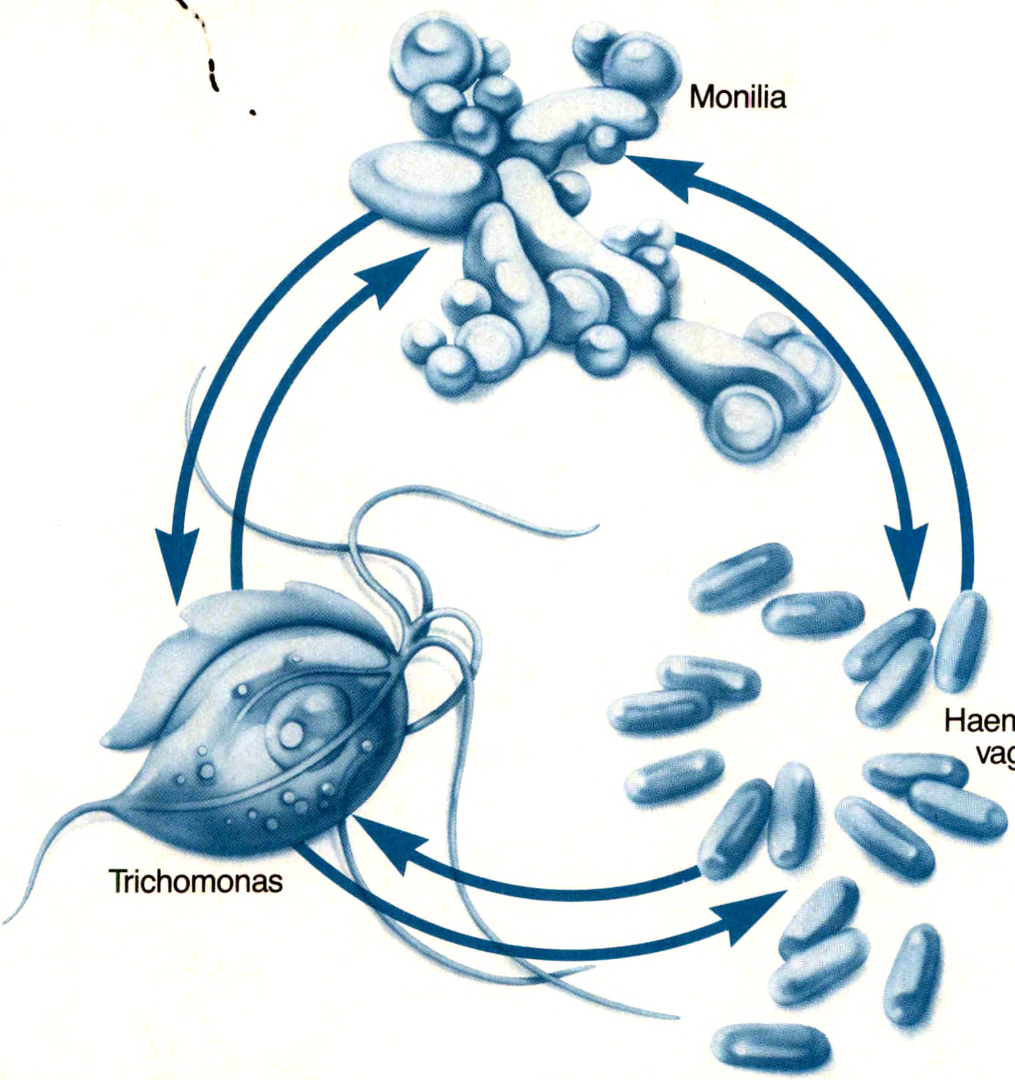

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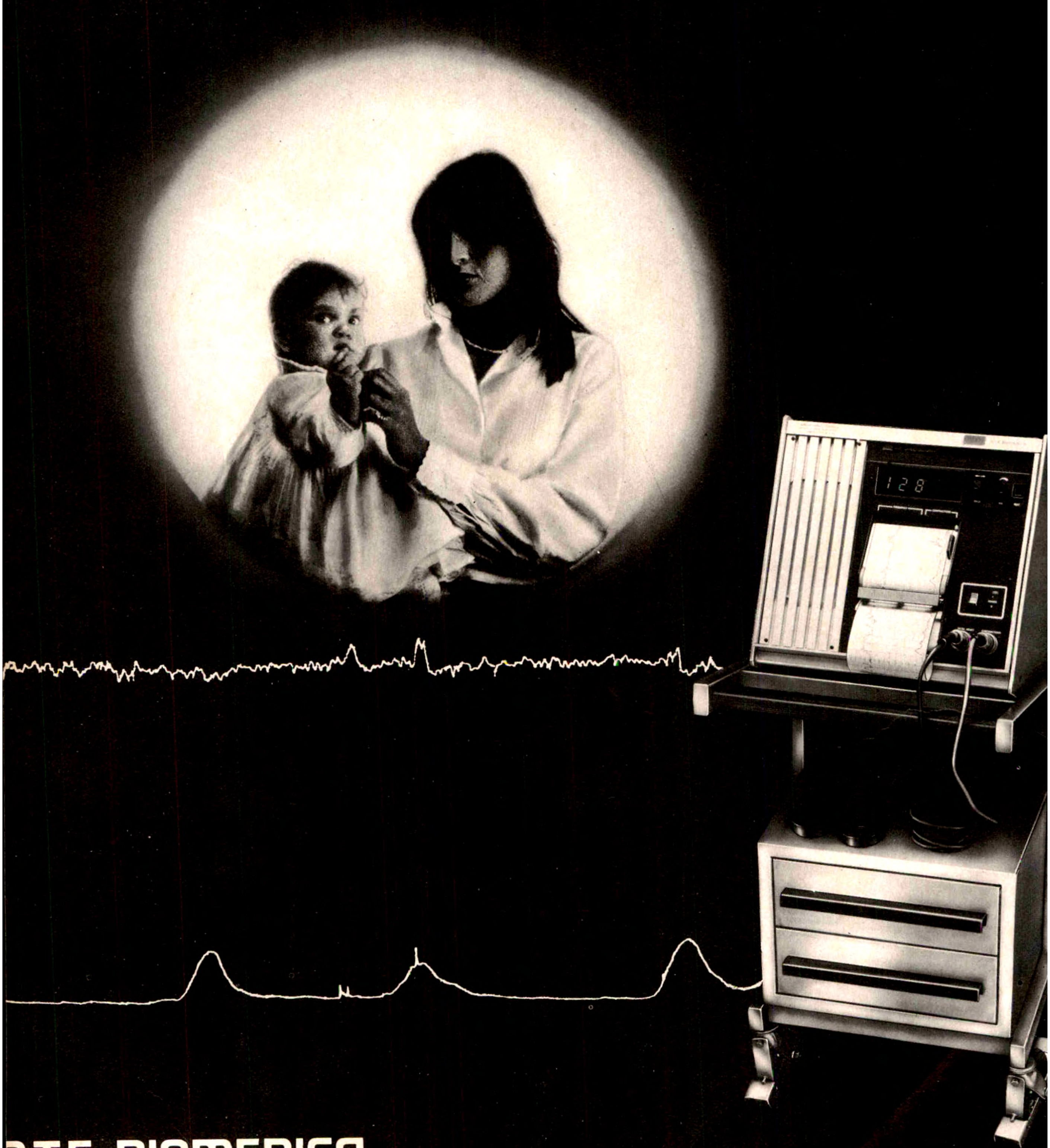

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Vol. 138, No. 8, December 15, 1980. The American Journal of Obstetrics and Gynecology is published semimonthly by The C. V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63141. POSTMASTER: Send address changes to above address.

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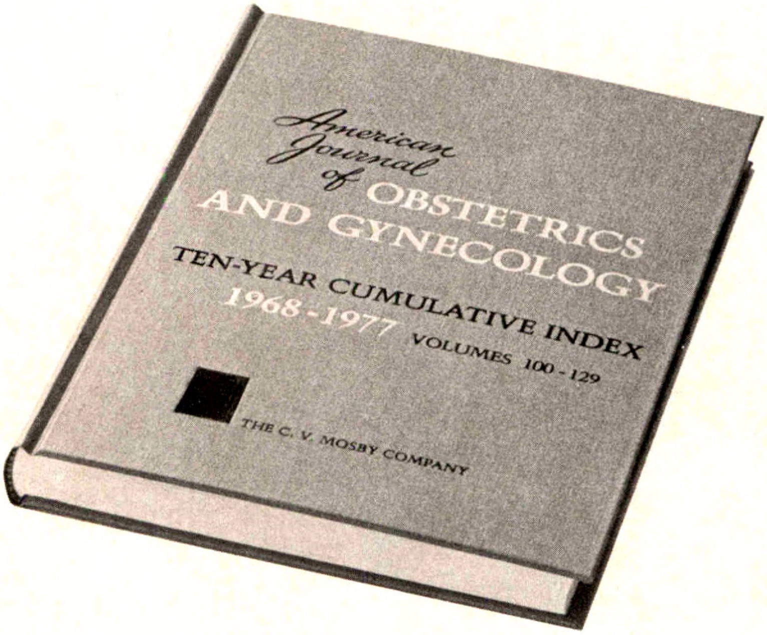
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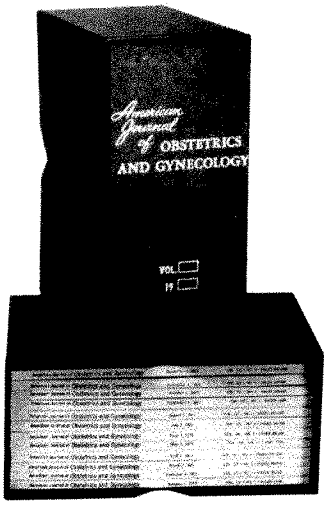

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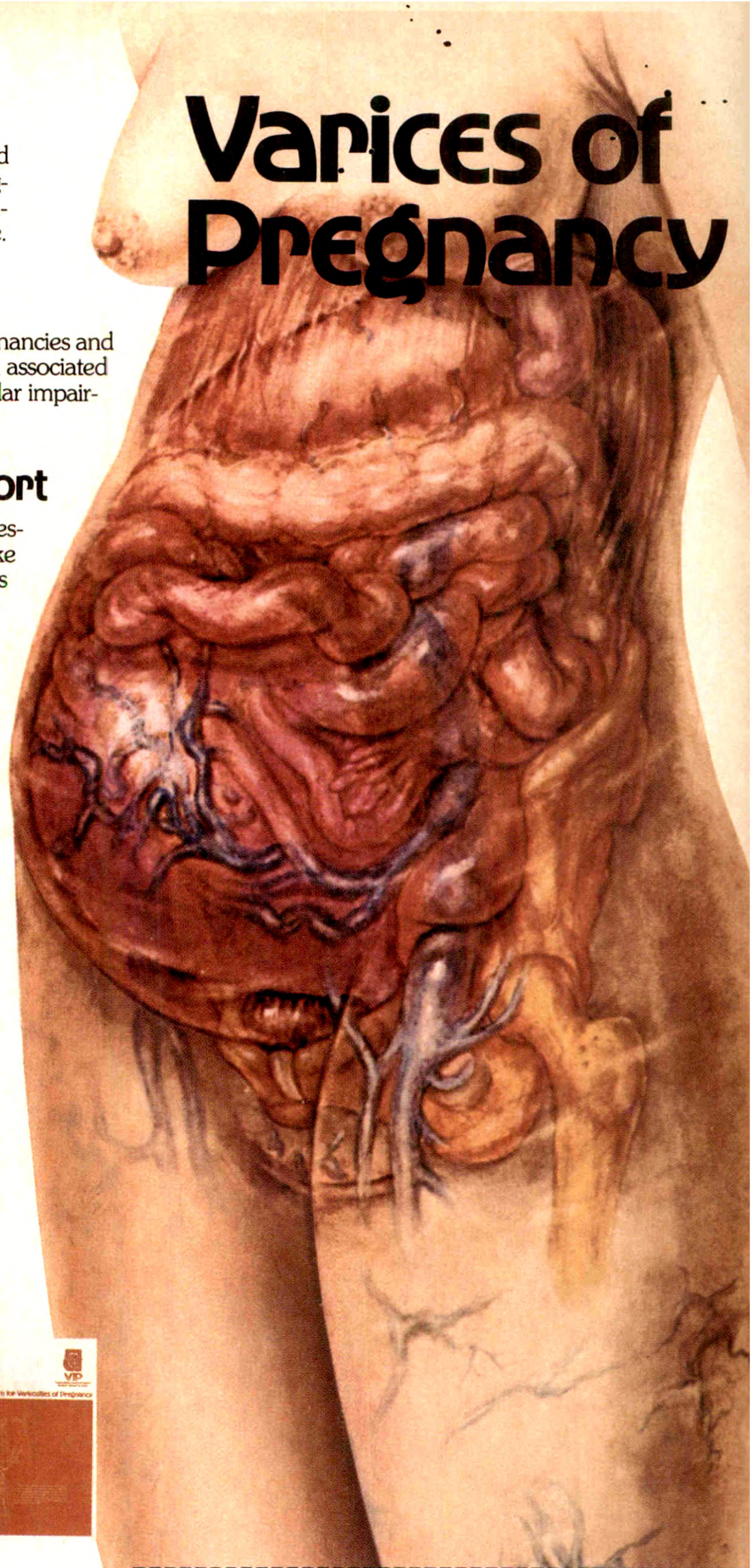
Varices of Pregnancy



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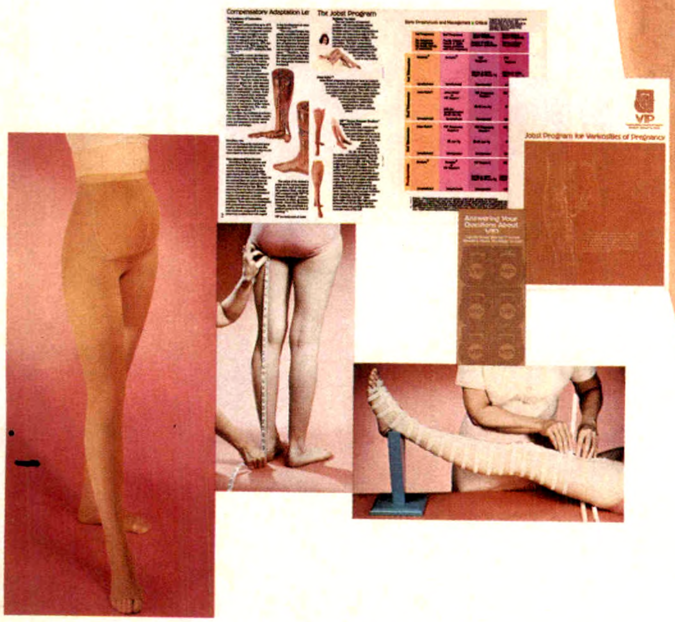
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. Fegan G: *Varicose Veins–Compression Sclerotherapy.* London, Wm Heinemann Medical Books Ltd, 1967.
. Thompson NW: The diagnosis and treatment of varicose veins. *Univ Mich Med Cntr J* 31 (1):11, 1965.

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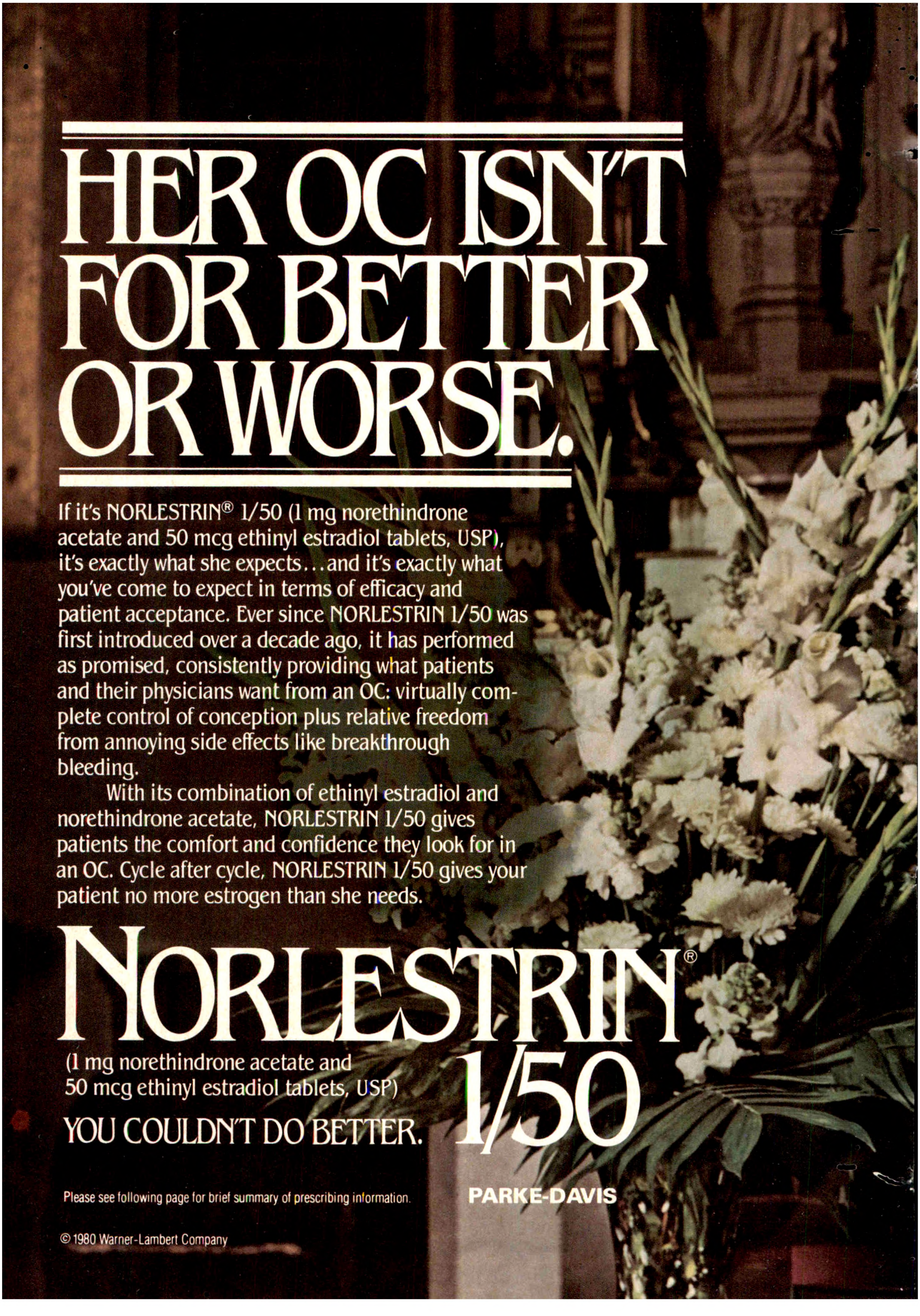

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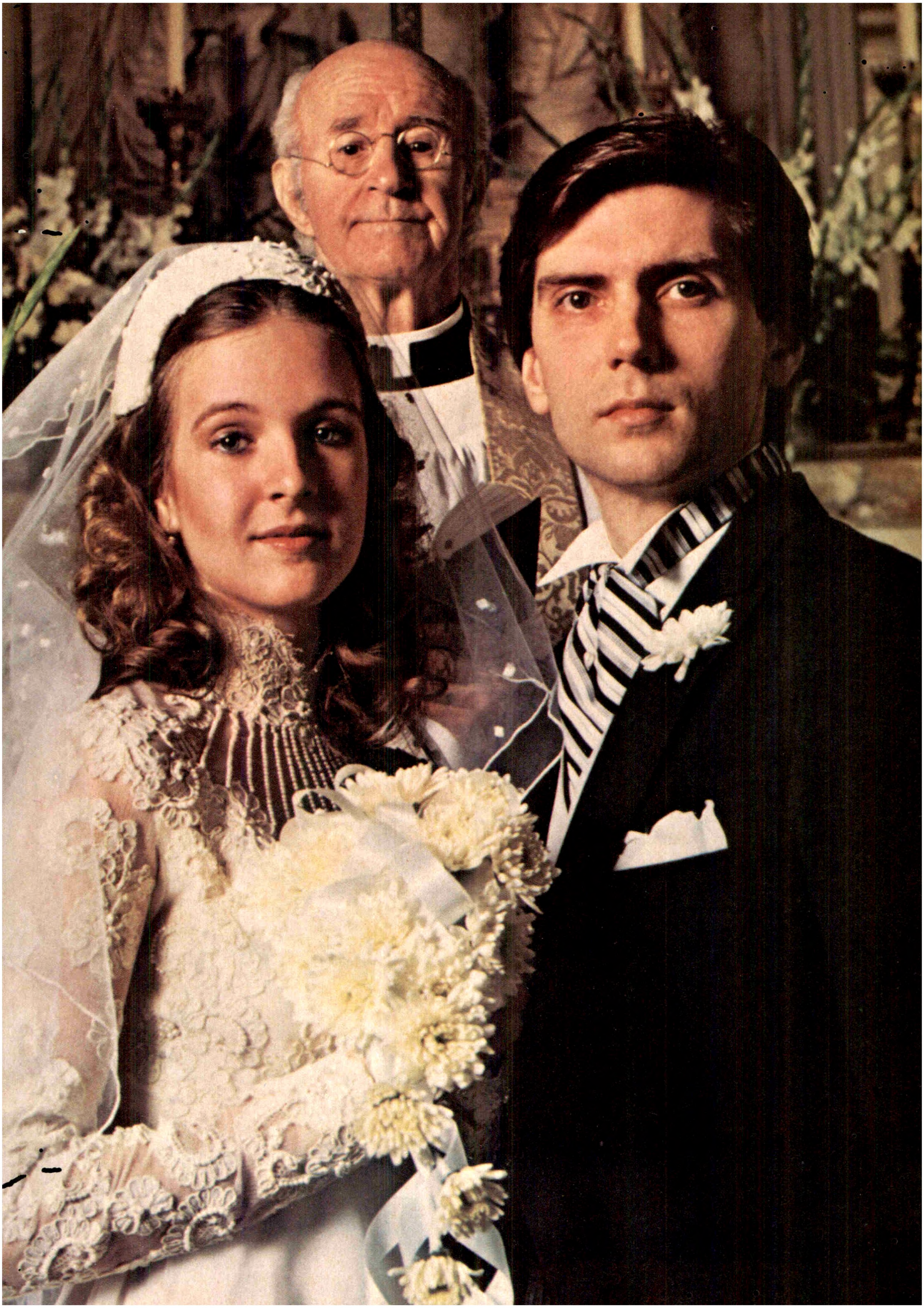

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Each yellow tablet contains: norethindrone acetate (17 alpha-ethinyl-19-nortestosterone acetate), 1 mg; ethinyl estradiol (17 alpha-ethinyl-1,3,5(10)-estratriene-3, 17 beta-diol), 50 mcg.

DESCRIPTION

Norlestrin 1/50 Products are progestogen-estrogen combinations.

INDICATIONS AND USAGE

Norlestrin 1/50 Products are indicated for the prevention of pregnancy in women who elect to use oral contraceptives as a method of contraception.

In clinical trials with Norlestrin 1/50 involving 25,983 therapy cycles, there was a pregnancy rate of 0.05 per 100 women years.

Dose-related risk of thromboembolism from oral contraceptives: Studies have shown a positive association between the dose of estrogens in oral contraceptives and the risk of thromboembolism. It is prudent and in keeping with good principles of therapeutics to minimize exposure to estrogen. The oral contraceptive prescribed for any given patient should be that product which contains the least amount of estrogen that is compatible with an acceptable pregnancy rate and patient acceptance.

CONTRAINDICATIONS

1. Thrombophlebitis or thromboembolic disorders
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders
3. Cerebral vascular or coronary artery disease
4. Known or suspected carcinoma of the breast
5. Known or suspected estrogen-dependent neoplasia
6. Undiagnosed abnormal genital bleeding
7. Known or suspected pregnancy (See WARNING No. 5.)

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, and hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well-established. Studies have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic.

Cerebrovascular disorders: In a collaborative study in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers, and the risk of thrombotic stroke was 4.0 to 9.5 times greater.

Myocardial infarction: An increased risk of myocardial infarction associated with oral contraceptives has been reported confirming a previously suspected association. These studies found that the greater the number of underlying risk factors (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) for coronary artery disease, the higher the risk of developing myocardial infarction, regardless of whether the patient was an oral contraceptive user or not. Oral contraceptives, however, were found to be a clear additional risk factor.

It has been estimated that users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. Oral contraceptive users who are smokers have about a fivefold increased risk of fatal infarction compared to users who do not smoke, but about a tenfold to twelvefold increased risk compared to nonusers who do not smoke. The amount of smoking is also an important factor.

Risk of dose: In an analysis of data, British investigators conluded that the risk of thromboembolism including coronary thrombosis is directly related to the dose of estrogen used in oral contraceptives; however, the quantity of estrogen may not be the sole factor involved.

Estimate of excess mortality from circulatory diseases: The risk of diseases of the circulatory system is concentrated in older women, in those with a long duration of use, and in cigarette smokers.

A study of available data from a variety of sources concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of oral contraceptives in women over 40 who smoke.

The risk of thromboembolic and thrombotic diseases associated with oral contraceptives increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of preeclamptic toxemia, and especially by cigarette smoking.

The physician and the patient should be alert to the earliest manifestations of thromboembolic and thrombotic disorders. Should any occur or be suspected, the drug should be discontinued immediately.

A fourfold to sixfold increased risk of postsurgery thromboembolic complications has been reported in users. If feasible, oral contraceptives should be discontinued at least four weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

2. *Ocular lesions:* Neuro-ocular lesions, such as optic neuritis or retinal thrombosis, have been associated with the use of oral contraceptives. Discontinue the oral contraceptive if there is unexplained sudden or gradual, partial, or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions.

3. *Carcinoma:* Long-term continuous administration of estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina, and liver.

In humans, an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women has been reported. However, there is no evidence suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only oral contraceptives.

Studies found no evidence of increase in breast cancer in women taking oral contraceptives; however, an excess risk in users with documented benign breast disease was reported.

There is no confirmed evidence of an increased risk of cancer associated with oral contraceptives. Close clinical surveillance of users is, nevertheless, essential. In cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer, or who have breast nodules, fibrocystic disease, or abnormal mammograms, should be monitored with particular care.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with oral contraceptives. Because hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage, they should be considered in women presenting abdominal pain and tenderness, abdominal mass, or shock.

A few cases of hepatocellular carcinoma have been reported in women taking oral contraceptives. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Usage in or Immediately Preceding Pregnancy; Birth Defects in Offspring, and Malignancy in Female Offspring:* During early pregnancy, female sex hormones may seriously damage the offspring.

An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of oral contraceptives in pregnancy.

There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing oral contraceptives.

Pregnancy should be ruled out before continuing an oral contraceptive in any patient who has missed two consecutive menstrual periods. If the patient has not adhered to the schedule, the possibility of pregnancy should be considered at the time of the first missed period, and oral contraceptives should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus and the advisability of continuation of the pregnancy should be discussed.

Women who discontinue oral contraceptives with the intent of becoming pregnant should use an alternate form of contraception for a period of time before attempting to conceive.

Administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of oral contraceptives.

7. *Carbohydrate and Lipid Metabolic Effects:* Because decreased glucose tolerance has been observed in a significant percentage of patients, prediabetic and diabetic patients should be carefully observed while receiving oral contraceptives.

An increase in triglycerides and total phospholipids has been observed.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving oral contraceptives. The prevalence in users increases with longer exposure. Age is also strongly correlated with development of hypertension. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure.

9. *Headache:* Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of oral contraceptives.

10. *Bleeding Irregularities.* Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing oral contraceptives. In breakthrough bleeding, nonfunctional causes should be borne in mind. In undiagnosed abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy.

Women with a past history of oligomenorrhea or secondary amenorrhea, or young women without regular cycles should be advised that they may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of oral contraceptives.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast Feeding:* Oral contraceptives may interfere with lactation. Furthermore, a small fraction of the hormonal agents in oral contraceptives has been identified in the milk of mothers receiving these drugs.

PRECAUTIONS

1. A complete medical and family history should be taken prior to the initiation of oral contraceptives. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen, and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, oral contraceptives should not be prescribed for longer than one year without another examination.
2. Preexisting uterine leiomyomata may increase in size.
3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.
4. Oral contraceptives may cause fluid retention and should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated.
5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice. If jaundice develops, the medication should be discontinued.
6. Steroid hormones may be poorly metabolized and should be administered with caution in patients with impaired liver function.
7. Users may have disturbances in normal tryptophan metabolism, which may result in a relative pyridoxine deficiency.
8. Serum folate levels may be depressed.
9. The pathologist should be advised of oral contraceptive therapy when relevant specimens are submitted.
10. Certain endocrine and liver function tests and blood components may be affected.

(a) Increased sulfobromophthalein retention. (b) Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. (c) Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone. (d) Decreased pregnanediol excretion. (e) Reduced response to metyrapone test.

Drug interactions: Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin and an association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and ampicillin.

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with oral contraceptives: thrombophlebitis; pulmonary embolism; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; benign hepatomas; congenital anomalies.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, eg, retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10% or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms; breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma; breast changes; change in weight; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately post partum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature; intolerance to contact lenses.

The following adverse reactions have been reported and the association has been neither confirmed nor refuted: premenstrual-like syndrome; cataracts; changes in libido; chorea; changes in appetite; cystitis-like syndrome; headache; nervousness; dizziness; hirsutism; loss of scalp hair; erythema multiforme; erythema nodosum; hemorrhagic eruption; vaginitis; porphyria.

Special Notes on Administration

Norlestrin Fe 1/50 (norethindrone acetate and ethinyl estradiol tablets, USP) and Norlestrin 28 1/50—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after the brown ferrous fumarate or white inert tablets have been started.

Norlestrin 21 1/50—Menstruation usually begins two or three days, but may begin as late as the fourth or fifth day, after discontinuing medication.

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PD-JA-0270-1-P (3-80)

American Journal of Obstetrics and Gynecology

volume 138 *number* 8 DECEMBER 15, 1980

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CLINICAL OPINION

This section reports opinion on the handling of clinical situations, i.e., the clinical diagnosis and management of certain disease entities. Papers should range from eight to twenty typed pages, including illustrations, tables, and figures which clarify the author's management. References are limited to sixteen citations. Mail to Frederick P. Zuspan, M.D., Editor.

Consideration of blood loss at delivery as percentage of estimated blood volume

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It is recommended that blood loss at delivery be evaluated in terms of percentage of estimated blood volume rather than in terms of volume only. Estimated blood volume in milliliters per kilogram may be calculated by the following formula:

$$\text{Blood volume (ml/kg)} = \frac{24}{0.86 \times \text{hematocrit}} \times 100.$$

Percentage of total blood volume lost at delivery may be calculated as follows:

$$\text{Percentage of total blood volume lost} = \frac{0.036 \times \text{estimated blood loss} \times \text{observed hematocrit}}{\text{body weight}}.$$

It is recommended that postpartum hemorrhage be defined as a loss of blood at delivery of greater than 15% of the total estimated blood volume. (AM. J. OBSTET. GYNECOL. 138:1117, 1980.)

OBSTETRIC RESIDENTS currently record blood loss at delivery in milliliters. Also postpartum hemorrhage is defined in obstetric textbooks as a loss of a volume of blood, be it 500 or 1,000 ml. This concept can be misleading because a certain blood loss in a patient with a large blood volume might be insignificant, whereas the same blood loss in a patient with a small blood volume might be highly significant. It would seem to be much more pertinent to evaluate and record blood loss at delivery as a percentage of estimated blood volume.

Blood volume is composed of two components—plasma volume and red blood cell volume. Of these two, estimates of red blood cell volume would appear to be much more consistent than estimates of plasma volume. In two independent studies (with radioisotope techniques) Berlin and associates[1] reported a red blood cell volume of 24.7 ml/kg and Gahres and associates[2] reported a value of 24.0 ml/kg.

Gahres and associates also showed that the mean red blood cell volume, expressed as milliliters per kilogram, was consistent throughout a range of patient weights

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0002-9378/80/251117+03$00.30/0 © 1980 The C. V. Mosby Co.

Table I. Comparison of direct blood volume determinations and estimation by simplified formula

Patient No.	*Determination No.*	*Observed hematocrit*	*Blood volume (ml/kg) (Statzer[5])*	*Estimated blood volume (ml/kg*)*	*Percent difference*
1	1	34.7	70	81	+16
	2	36.6	68	76	+12
	3	36.6	67	76	+13
2	1	39.4	77	71	− 8
	2	39.4	80	71	−11
3	1	31.2	97	90	− 7
	2	32.0	99	87	−12
	3	28.2	94	99	+ 5
4	1	37.6	77	74	− 4
	2	36.6	71	76	+ 7
	3	37.6	73	74	+ 1
	4	37.6	69	74	+ 7
5	1	34.7	76	81	+ 7
	2	37.6	77	74	− 4
	3	37.6	75	74	− 1
6	1	33.8	80	82	+ 3
	2	32.9	81	85	+ 5
7	1	29.1	73	96	+32
	2	29.1	73	96	+32

$$*\text{Blood volume (ml/kg)} = \frac{24}{0.86 \times \text{hematocrit}} \times 100.$$

Table II. Consideration of 1,000 ml blood loss in two hypothetical patients

Patient No.	*Body weight (kg)*	*Observed hematocrit*	*Estimated blood loss at delivery (ml)*	*Percentage of total blood volume lost**	*Postpartum hemorrhage*
1	80	30	1,000	13.5	No
2	50	40	1,000	28.8	Yes

$$*\text{Percentage of total blood volume lost} = \frac{0.036 \times \text{estimated blood loss} \times \text{observed hematocrit}}{\text{body weight}}.$$

from 50 to 100 kg. Therefore, in an effort to estimate blood volume at delivery the most reasonable starting point would appear to be an assumption of a red blood cell volume of 24 ml/kg. In order to convert this value to blood volume, the hematocrit must be used. Chesley[3] has shown that the observed hematocrit has two inherent errors. First, the hematocrit should be corrected for plasma trapped in the volume of red cells. This varies with the centrifugal force and duration of centrifugation but averages about 5% with usual methods of hematocrit determination. Second, the "whole-body hematocrit" is less than the observed hematocrit because the blood in the minute vessels has a lower percentage of red blood cells than blood drawn from a peripheral vein. It has been shown that the whole-body hematocrit is 91% of the observed hematocrit. The observed hematocrit may then be corrected by multiplying by 0.95, then 0.91, or by using a single multiplication factor of 0.86.

Therefore, the most logical method for estimation of blood volume would appear to be to use an assumed red blood cell volume and the corrected hematocrit. This concept is supported by the data of Lund and Donovan,[4] who showed that the hematocrit varies inversely with the plasma volume and that the hematocrit usually reflects changes in plasma volume and not changes in red blood cell volume.

In order to test the accuracy of these calculations, the data of Statzer[5] are used. Statzer determined blood volume with the use of iodinated human serum albumin 19 times in seven patients prior to delivery and also reported hematocrit values and body weight. All but one of the determinations were made within 4 weeks of delivery. In the one other case evaluation was done 16 weeks prior to delivery. The data are shown in Table I, along with estimated blood volume calculated by the formula:

$$\text{Blood volume (ml/kg)} = \frac{24}{0.86 \times \text{hematocrit}} \times 100.$$

As can be seen, with the exception of Patient No. 7, the estimated blood volume is reasonably close to the measured blood volume. In fact, in averaging the percentage differences in the 17 determinations (excluding Patient No. 7), the estimated blood volume is 2% higher than the measured blood volume. In examining closely the data on Patient No. 7, the plasma volume was normal, the hematocrit was low, and the red blood cell volume was low (18 ml/kg). One could make a strong case for a true anemia in this patient.

Therefore, I would like to propose that the following should be done for each patient at delivery:

1. The patient's weight in kilograms should be recorded and the venous blood hematocrit should be determined.
2. The hematocrit should be corrected by multiplying by 0.86.
3. The blood volume in milliliters per kilogram should be estimated by dividing the corrected hematocrit into 24.
4. The total estimated blood volume should be determined by multiplying blood volume in milliliters per kilogram by the body weight.
5. The blood loss at delivery should be recorded in milliliters.
6. The blood loss at delivery should be calculated as a percentage of total estimated blood volume.
7. Postpartum hemorrhage should be defined as a loss of blood at delivery of greater than 15% of the total estimated blood volume.

On a more practical basis all of the above calculations may be simplified into the following formula:

$$\text{Percentage of total blood volume lost at delivery} = \frac{0.036 \times \text{estimated blood loss} \times \text{observed hematocrit}}{\text{body weight}}.$$

Table II illustrates the consideration of a 1,000 ml blood loss in two hypothetical patients. It might be expected that Patient No. 1 will tolerate the blood loss quite well, whereas Patient No. 2 might show signs and symptoms of acute hemorrhage.

Finally, it should not be forgotten that the reliability of these calculations is totally dependent upon a normal red blood cell volume. Conditions such as true anemias will cause overestimation of the blood volume and conditions which stimulate erythropoiesis beyond that of normal pregnancy will result in underestimation of blood volume. Despite this fact and the normal, expected patient-to-patient variation in red blood cell volume, this method of evaluation of blood loss at delivery should be a considerable improvement over the currently accepted method.

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GYNECOLOGY

Benign cystic teratomas in postmenopausal women

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Postmenopausal benign cystic teratomas occurred in 16 women in a 6-year period. In this group the incidence of malignancy was 19%, and the incidence of bilaterality was 20%. Marked collagenization of the tissue of the teratoma indicated an "end-stage" process. The high incidence of malignancy in this group of patients is an indication for vigorous surgical staging for extent of disease. (AM. J. OBSTET. GYNECOL. 138:1120, 1980.)

BENIGN CYSTIC teratomas (dermoid cysts) are ovarian neoplasms derived from germ cells and composed of mature tissues representing two or more of the embryonic germ cell layers. The "cyst" almost always contains hair, desquamated stratum corneum, and sebaceous material derived from the "skin" that lines the cavity. Usually benign, dermoid cysts may be associated with more malignant germ cell tumors or may exhibit secondary malignancy after initial benign growth. They represent 15% to 20% of all benign ovarian tumors and 10% of all ovarian tumors.[1] The peak incidence is in the third and fourth decades of life. Classically, then, a tumor of young women, they are surgical surprises when encountered in postmenopausal women and seem to be worthy of review.[1, 2]

Material and methods

A 6-year period from January 1, 1973, through December 31, 1978, was selected for analysis. The files of The Johns Hopkins Hospital and the Sinai Hospital of Baltimore were reviewed and all cases of benign cystic teratoma were collected. Referred cases were excluded. Those cases which occurred in women who had experienced at least 1 year of amenorrhea were then identified and reviewed pathologically to confirm the diagnosis. The preoperative clinical studies were collected and collated.

Results

Three hundred and nine cases of benign cystic teratomas were identified in this 6-year period. The age range of the patients was 2 to 80 years. The largest group of cases (130 patients, 42%), occurred in the age group of 20 to 29 years. Sixteen patients (5%) were postmenopausal. The age range of these patients was 49 to 80 years, with a mean of 49.

Clinical features.

Presenting complaint. Thirteen of 16 patients were admitted for evaluation of an asymptomatic pelvic mass. In three patients the teratoma was discovered incidentally at the time of operation for other gyneco-

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Received for publication January 31, 1980.
Revised July 16, 1980.
Accepted July 21, 1980.

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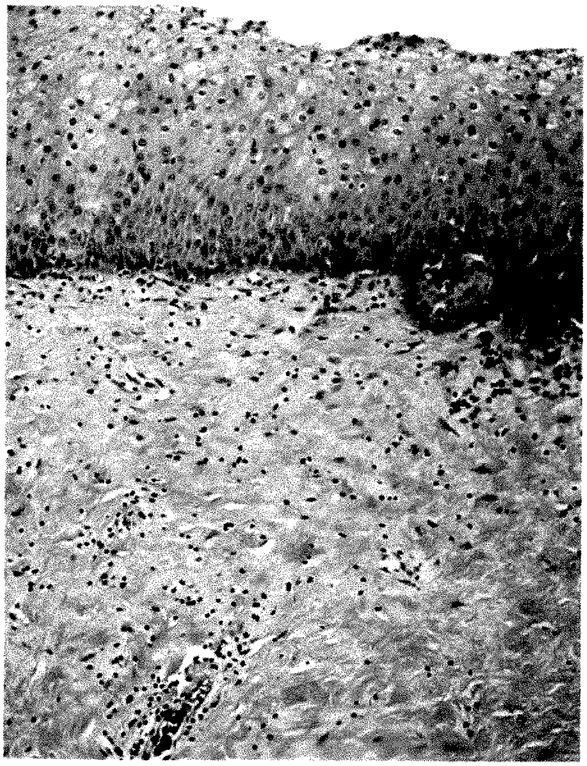

Fig. 1. Squamous epithelium (epidermis) overlies a layer of dense collagen that is relatively acellular.

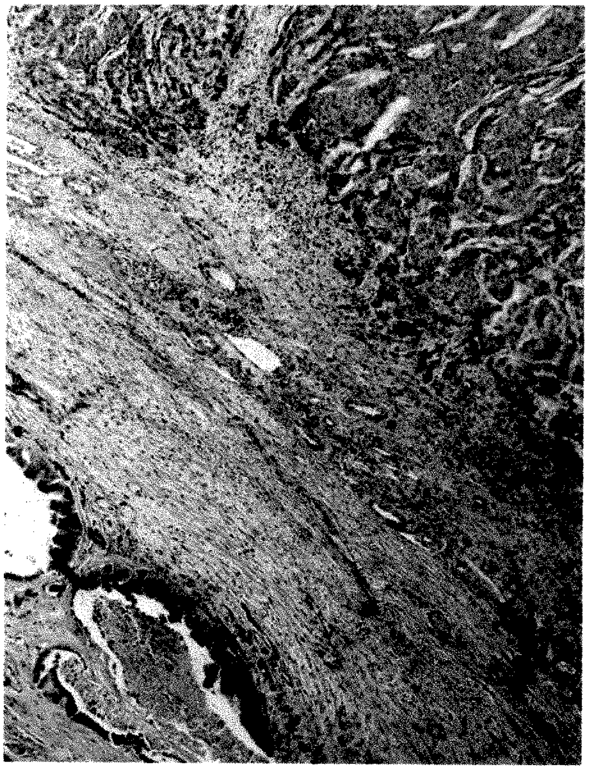

Fig. 2. Squamous cell carcinoma is seen in the upper corner of the photograph, and an adenocarcinoma below. This field illustrates the point at which the tumors were in closest proximity.

logic conditions, i.e., invasive carcinoma of the cervix (one) and uterine leiomyomas (two).

Preoperative evaluation. Eleven patients underwent preoperative x-ray examinations. Seven of 10 intravenous pyelograms disclosed a pelvic mass, only one of which showed calcification within the mass. Two of the three barium enemas disclosed evidence of a pelvic mass with calcifications. Of three sonograms which were recorded, one was read as suggesting calcification, and another as showing a "15 by 19 cm mass with fluid levels and a central mass probably representing a benign cystic teratoma." The four examinations which demonstrated calcifications were performed in four different patients. The calcifications were confirmed histologically in all the cases, except that revealed by the intravenous pyelogram.

No patient had a preoperative clinical diagnosis of benign cystic teratoma.

Pathologic features.

Size. The dermoid cysts detected incidentally were small, with the largest one being 6 by 5.5 by 4.5 cm, whereas among those patients who were admitted to the hospital with a diagnosis of pelvic mass, one cyst was as large as 18 by 15 by 9 cm.

Bilaterality. Of the 16 patients, one had previously undergone an oophorectomy, but three (20%) of the other 15 had bilateral dermoid cysts.

Histopathologic findings. In 12 cases, "skin" could be identified. This consisted of stratified squamous epithelium with epidermal appendages. However, in all cases the dermis was markedly scarred, having been almost totally replaced by dense collagen with obliteration of the loose reticular tissue of the normal dermis (Fig. 1).

In five cases there was respiratory epithelium, and in two, thyroid tissue. Secondary neoplasms were present in three cases: one in which the mucinous epithelium was consistent with a borderline mucinous cystadenocarcinoma, a second in which the stroma demonstrated solid proliferation of small uniform cells containing nonargentaffin but argyrophilic granules, interpreted as a stromal carcinoid, and a third in which both a squamous cell carcinoma and adenocarcinoma were present (Fig. 2). The patients with the mucinous cystadenocarcinoma and stromal carcinoid were alive and well 36 and 24 months after diagnosis. The patient with

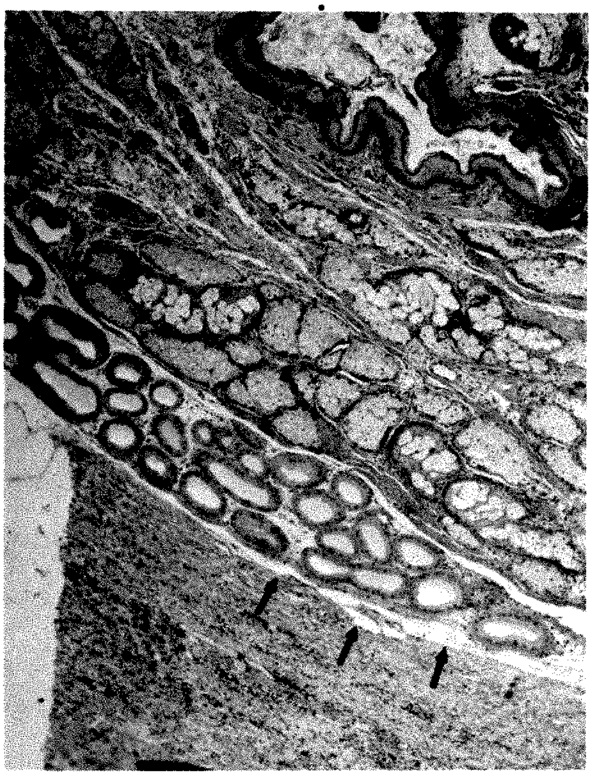

Fig. 3. Squamous epithelium (epidermis) rests on a layer of dermis that contains multiple appendages, muscle, and fibroblasts. Arrows illustrate the line of demarcation between the neoplasm and the normal ovary.

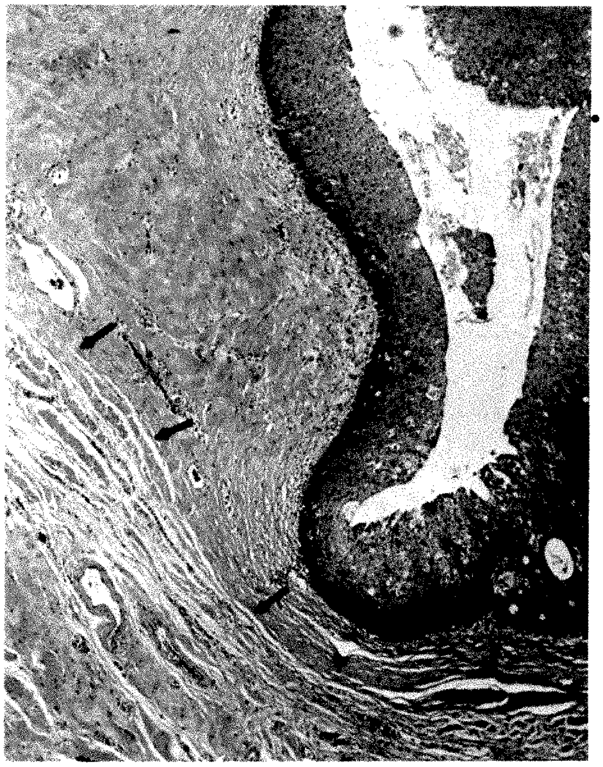

Fig. 4. Arrows illustrate the line of demarcation between the dermis and the normal ovary. In this case the dermis contains no appendages, and the loose reticular tissue has been connected to a layer of dense collagen.

squamous cell carcinoma died of a primary adenocarcinoma of the colon.

Comment

Dermoid cysts are routinely lined by stratified squamous epithelium with appendages that are typical of the skin. Classic descriptions are less adequate on the point, but this lining is also routinely supported by a well-defined dermis that contains the epidermal appendages, as well as fat, muscle, and other connective tissue (Fig. 3). Because this tissue is clearly demarcated from the surrounding ovary, these tumors can usually be easily shelled from the surrounding ovary. This tissue is dramatically apparent in postmenopausal dermoids cysts, since it has uniformly been replaced by dense scarlike tissue which is largely collagen and appears to be composed of scattered fibroblasts and collagen (Fig. 4). We interpret this to indicate the prolonged presence of these tumors within the ovary, thus suggesting that they reposed within the young patient and were not detected until she reached menopause.

A further point of clinical interest is that dermoid cysts were uniformly not suspected preoperatively, although there were some preoperative clues, i.e., calcifications within a pelvic mass.

The rate of bilaterality, 20%, in our material corresponds to a range of 10% to 20% reported in previous studies.[1, 3] This high rate of bilaterality is additional evidence that this is an "end stage" disease process when detected in postmenopausal women.

Malignant degeneration occurred in three (1%) of the 309 cases studied, which is comparable to an incidence of 1% to 2% reported in previous studies.[3-6] In the present study, all cases of malignancy (an incidence of 19%) occurred in postmenopausal women. In a review of 351 cases of malignancy arising in benign cystic teratomas, the ages of the patients were available for analysis in 318.[3-19] Their age range was 9 to 80 years. One hundred and eighty-one (57%) of the 318 patients were 50 years of age or older. Thus, it would appear that most secondary malignancies in cases of benign cystic teratomas arise in women over age 50.

The occurrence of a benign cystic teratoma in the postmenopausal woman is thus of concern, and all cases should be managed as if they were potentially malignant ovarian tumors. Thorough staging procedures to evaluate the extent of disease should be carried out, as well as extensive sampling of the dermoid cyst.

Three hundred nine cases of benign cystic teratomas were reviewed. The peak incidence was in the age group 20 to 29 years, with 5% of the population being postmenopausal. All three cases of a malignancy that arose from a benign cystic teratoma occurred in the postmenopausal group. Aggressive surgical staging is advocated in all cases of postmenopausal benign cystic teratomas because of the high incidence of malignancy.

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Second-look operations in ovarian cancer

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One hundred eighty-six patients with epithelial tumors of the ovary who were managed at the M. D. Anderson Hospital and Tumor Institute underwent an exploratory operation ("second-look" operation) to assess the status of the cancer. Fifty-eight patients were found to have no evidence of disease (NED) and chemotherapy was discontinued. Eight patients with advanced ovarian cancer had NED 5 or more years after negative second-look operations and were probably cured by chemotherapy. Seven patients who were found to have cancer at second-look operations had a change in management as a result of the operation and had NED 5 or more years later. The most important factors correlating with negative second-look operations were the stage of cancer, the amount of residual tumor left at the initial operation, and the number of courses of chemotherapy administered prior to the second-look operation. Survival after positive second-look operations varied directly with the volume of tumor found at the time of operation and the amount of tumor left behind at the second-look operation. Patients who were treated with radiation therapy had a poorer survival rate than those who were treated with chemotherapy after positive second-look operations. (AM. J. OBSTET. GYNECOL. 138:1124, 1980.)

EXPLORATORY laparotomy after a planned program of chemotherapy, i.e., the "second-look" operation, has been incorporated into the management of patients with epithelial tumors of the ovary at the M. D. Anderson Hospital since 1960. The purpose of the operation is to assess the status of the patient's tumor in order to plan future treatment and to remove, if possible, any tumor that is found. This report reviews the experience at the M. D. Anderson Hospital with second-look operations in the management of patients with ovarian epithelial tumors to determine whether the operation has a significant role in their management.

Material and methods

One hundred ninety-two patients had second-look operations after an interval of chemotherapy during the 14-year interval between 1960 and 1974. Six patients who were thought to have recurrent tumor after irradiation therapy, but in whom laparotomy did not prove the existence of cancer before chemotherapy was begun, will be excluded from the statistics. The stage of the tumors of the other 186 patients, according to the current International Federation of Gynaecology and Obstetrics (FIGO) classification, may be seen in Table I. Twenty-seven of the tumors were State I; 31 were Stage II; 105 were Stage III; and 23 were Stage IV. Most of the patients with Stage I and Stage II cancers were treated prophylactically and will not be included in the evaluation of second-look operations in the management of ovarian cancer. Histologically, the predominant tumor pattern was serous carcinoma (127), followed by unclassified epithelial (30), mucinous (16), and endometrioid (13). The histologic characteristics of the tumors were not reviewed for this report, and many of the patients with endometrioid tumors or mesonephroid tumors who were treated before 1970 were classified as having serous tumors. The tumor grade was known in 145 patients. One patient had a tumor of low malignant potential, 21 patients had grade 1 lesions, 49 patients had grade 2 lesions, and 76 had grade 3 lesions. Thirty-nine patients had ungraded tumors. The more undifferentiated tumors tended to be associated with more advanced stages. Fifty-nine of the 76 (78%) patients with grade 3 tumors had stage III or Stage IV lesions.

Thirteen of 128 patients with Stage III or Stage IV cancer who underwent a second-look operation had no

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Received for publication November 26, 1979.

Revised July 16, 1980.

Accepted July 22, 1980.

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residual tumor at the time of the original operation. Thirty-one patients had residual tumor less than 2 cm in diameter, and 83 patients had residual tumor greater than 2 cm in diameter. The volume of residual cancer was not stated in one patient's chart.

Single-agent chemotherapy was administered to 173 of the 192 patients who underwent second-look operations. Melphalan was used in 167 patients, Adriamycin in two, hexamethylmelamine in two, and 5-fluorouracil in one. One patient who was treated with oral cyclophosphamide for 27 months prior to being referred underwent a second-look operation upon admission to the M. D. Anderson Hospital. Nineteen patients were treated with a combined chemotherapy which consisted of actinomycin-D, 5-fluorouracil, and cyclosphosphamide (AcFuCy), prior to the second-look operation.

The timing of the second-look operation changed as experience was gained in the chemotherapeutic management of patients with advanced cancer. The first several patients underwent a second-look operation when a significant change in the palpable tumor suggested that a previously unresectable tumor could be excised. More recently, second-look operations were performed when a patient developed prolonged depression of bone marrow after chemotherapy and there was concern that the tumor might begin to grow again before the next course of chemotherapy could be instituted. Since this plan of treatment did not prove to be beneficial for many patients, it has slowly evolved until, at the present time, only those patients who have received 12 or more cycles of chemotherapy are subjected to second-look operations. Patients treated with Adriamycin received a total dose of 540 mg per square meter of body surface area before undergoing the second-look operation.

The surgical technique used at the time of second-look operations has changed significantly during the period of study. The present plan is to perform a laparotomy through a vertical midline incision from the symphysis to approximately 6 cm above the umbilicus, and then obtain cytologic washings from the pelvis and each lateral paracolic space and multiple biopsy specimens of the abdominal and pelvic peritoneum if no gross tumor is present. An attempt is made to completely excise all residual tumor if gross tumor is present. The multiple sites of biopsy include the known sites of residual tumor recorded at the initial operation and usually include the lateral pelvic peritoneum, cul-de-sac peritoneum, and anterior bladder peritoneum, both lateral paracolic spaces, and the inferior surface of the diaphragm. If ovaries or uterus is present, they are removed, and the omentum is removed routinely. The bowel serosa and mesentery are carefully inspected, and biopsy specimens are taken from any irregularity, as well as a sample of para-aortic lymph nodes. From 25 to 30 biopsy specimens are obtained for study from most patients.

Table I. Stage of ovarian cancer

Stage	*No. of patients*
I	27
II	31
III	105
IV	23
Total	186

Table II. Stage versus findings at second-look operation

Stage	*Total No. of patients*	*No. of patients with NED*	*No. of patients with persistent cancer*	*% NED*
I	27	18	9	66.7
II	31	14	17	45.2
III	105	17	88	16.2
IV	23	9	14	39.1
Total	186	58	128	

During the first 10 years covered in this report, patients with tumor masses smaller than 2 cm after second-look operations were treated with whole-abdomen irradiation by the moving-strip technique, with additional irradiation to the pelvis, and patients with tumor masses larger than 2 cm were continued on chemotherapy. The complications and long-term survival rates of patients who were treated with irradiation after second-look operations did not justify this treatment.

Each patient's record was reviewed to ascertain the response to chemotherapy as determined at the second-look operation. These findings were categorized as (1) no evidence of disease (NED); (2) NED, but positive cytologic studies of peritoneal washings; (3) regression of the original tumor mass, with only microscopic tumor present in random biopsy specimens; (4) regression of the original tumor mass, but macroscopic tumor present; (5) no change in the volume of tumor since the initial operation; and (6) growth of the original tumor during chemotherapeutic treatment.

Results

Stage I. Eighteen of 27 patients (67%) underwent negative second-look operations, and nine patients were found to have persistent cancer (Table II). All but one patient had received melphalan, and the number of treatment courses received by the patients with negative second-look operations varied from 10 to 13. All patients who had negative second-look operations had NED 5 months to 10 years postoperatively.

Table III. Findings at second-look operation

Findings	*No. of patients*
NED	31
NED − + Cytology	0
Regression—Microscopic tumor	19
Regression—Macroscopic tumor	59
No change	17
Progression	16
Total	142

Table IV. Number of courses of melphalan versus negative findings (NED) at second-look operation

No. of courses of melphalan	*No. of patients with NED*	*No. of patients with persistent cancer*	*% NED*
2-9	7	41	14.6
10	8	21	27.6
11	0	2	0
12	8	12	40.0
13+	6	4	60.0

Among the nine patients who were found to have cancer at the time of the second-look operation, six had Stage IC cancer and three had Stage IB cancer. Eight of the nine patients had been treated with melphalan, but only two patients received 12 courses prior to the second-look operation. Chemotherapy was changed in five patients because cancer was found at the second-look operation, and one patient who had NED 35 months later had a negative third-look operation. A second patient underwent a negative third-look operation after a change in chemotherapy but developed a recurrence 39 months later. One patient was currently alive and receiving chemotherapy 9 months postoperatively. Two patients who continued to receive chemotherapy died 10 and 38 months postoperatively. Three patients received whole-abdomen irradiation by the moving-strip technique, with additional irradiation of the pelvis, after findings of persistent cancer at a second-look operation, and two had NED 36 and 98 months after that treatment. The other patient died of cancer 54 months after undergoing radiation therapy. One patient with persistent cancer was given no further treatment and died 13 months postoperatively.

Stage II. Fourteen of 31 patients (45%) underwent negative second-look operations (Table II). Nine of the 14 patients who were free of disease at the second-look operation had no residual tumor remaining at the original operation, two had residual tumor less than 2 cm in maximum diameter, and three had residual tumor greater than 2 cm. Three patients have survived 60 months or more without evidence of recurrent disease. Two of the latter patients had residual tumor masses greater than 2 cm at the time of the original operation and had grade 2 and grade 3 serous carcinoma. Six patients survived 36 to 58 months with NED after second-look operations. All but one patient received 9 to 12 courses of melphalan.

Eight of the 17 patients who had cancer at the time of the second-look operation were clinically free of disease prior to operation. Four of the eight had NED 6, 15, 29, and 83 months after a change in treatment. Nine patients with palpable tumor masses prior to the second-look operation died of disease 7 to 31 months (average, 15.9 months) after the second-look operation. Only one of the nine patients with palpable tumor masses and three of the eight patients with persistent cancer received 12 or more cycles of chemotherapy prior to operation.

Stage III. Seventeen of 105 patients (16%) with Stage III tumors were free of cancer at second-look operations (Table II). Two had NED 8 and 9 years after negative second-look operations. One patient was still living 37 months after a negative second-look procedure, and nine patients had NED 4 to 22 months after negative second-look operations. Four patients developed recurrent tumor 6, 7, 12, and 28 months postoperatively and died of the disease. The one other patient received 18 courses of melphalan, underwent a negative second-look operation, and died of acute leukemia 16 months postoperatively, with no evidence of ovarian carcinoma at postmortem examination.

Fifteen of the patients who had negative second-look operations were treated with melphalan, one with 5-fluorouracil, and one with cyclophosphamide. Eight of the 15 patients treated with melphalan received 12 or more courses of it, but the four patients who developed recurrent cancer after a negative second-look operation received only 4, 5, 7, and 10 courses of melphalan.

Forty-two of 88 patients who were found to have cancer at the second-look operation were clinically free of cancer immediately prior to operation. Eleven of these are alive and without evidence of cancer, at the time of this writing. Six had NED at 5 or more years (65 to 143 months). Four of these patients had a change in treatment because of the findings at the second-look operation, one patient continued on the same chemotherapy, and one patient underwent excision of the only known focus of residual tumor. The other five patients had NED 35 to 39 months after positive second-look operations.

Stage IV. Nine of 23 patients (39%) with Stage IV ovarian carcinoma were found to have NED at the time of the second-look operations (Table II). Three patients have survived more than 60 months after the second-look operation, and three have survived free of recurrent tumor 35, 38, and 43 months. Three patients developed recurrent tumor 7, 9, and 15 months after the second-look operations.

All of the patients with Stage IV cancers who had NED at the time of the second-look operations had been given melphalan. Six were given 10 to 35 courses of treatment. The patient who received 35 courses of melphalan prior to the second-look operation died 80 months postoperatively from acute granulocytic leukemia. Three patients who developed recurrent cancer received 4, 12, and 15 courses of melphalan; however, the patient who was given 12 courses of melphalan had a second-look operation that was inadequate by our present standards.

Five of the 14 patients with residual tumor that was found at a second-look operation had no palpable tumor masses prior to operation. There were no long-term survivors in this group.

In a review of the group of patients not treated prophylactically, i.e., the 14 Stage II patients with residual tumor left at the initial operation, as well as all Stage III and Stage IV patients, 31 of 142 patients had NED at the second-look operation (Table III). No patients had cytologic evidence of malignancy in abdominal washings with negative random biopsies. Nineteen patients had no gross evidence of disease, but were found to have microscopic disease in random biopsies. In several patients, only one biopsy site was positive for microscopic disease, despite multiple random biopsies. Tumor regression with macroscopic (visible) tumor was present in 59 patients. Seventeen patients demonstrated no change in the status of the tumor from the time of the initial operation. Sixteen patients showed definite progression of the disease as compared to the findings at the initial operation.

Seven of 48 patients (14.6%) who completed two to nine courses of melphalan were found to have NED at the second-look operation (Table IV). Eight of 29 patients (27.6%) who were given 10 courses of melphalan had NED, and 8 of 22 patients (36.4%) who received 11 or 12 courses of melphalan had NED at the second-look operation. Ten patients completed 13 or more courses of melphalan, and six (60%) had NED at the second-look operation.

The volume of residual tumor left at the initial operation correlated directly with the incidence of negative second-look operations. The survival rate for patients whose largest residual tumor mass was less than 2 cm was almost the same as that for patients who had no tumor remaining after the initial operation. Four of 13 patients (30.8%) who had NED at the time of the original operation had negative second-look operations. Twelve of 38 patients (31.5%) with less than 2 cm of residual tumor had negative second-look operations, and 15 of 90 patients (16.7%) with more than 2 cm of residual tumor had negative second-look operations.

Table V. Location of residual tumor at second-look operation

Site	*No. of patients*
Pelvic peritoneum	58
Omentum	57
Ovary*	53
Bowel serosa	38
Sigmoid colon	34
Abdominal gutters	23
Anterior abdominal peritoneum	21
Bowel mesentery	20
Pelvic retroperitoneum	18
Fallopian tubes	16
Para-aortic lymph nodes	15
Liver serosa	12
Uterus	11
Other	24

*Only 56 patients had ovaries at the time of the second-look operation.

The majority of nonprophylactically treated patients (19) with negative second-look operations had serous carcinoma, but only 18.8% of patients with serous carcinoma were free of tumor at the time of the second-look operation. The percentage of negative second-look operations was higher for mucinous (25%), endometrioid (37.5%), and unclassified adenocarcinoma (28%). The histologic grade of tumor was known in 112 of the 142 nonprophylactically treated patients. Two of 11 patients (18.2%) with grade 1 lesions, six of 33 patients (18.2%) with grade 2 lesions, and 18 of 68 patients (26.5%) with grade 3 lesions had NED at the time of the second-look operation.

The sites of persistent disease in all patients with positive second-look procedures are seen in Table V. The pelvic peritoneum, ovaries, and omentum were the most common sites of persistent disease; however, biopsy specimens of the para-aortic lymph nodes and the diaphragm were not obtained in most of the patients in this series. Fifty-six patients with Stage III or Stage IV cancer had ovaries left in place at the initial operation, and only three were free of cancer at the second-look operation. Only recently has visualization of the diaphragm via a sigmoidoscope or laparoscope been incorporated into the surgical routine at the time of a second-look operation. It is interesting that, in all patients in whom either gross or microscopic cancer was found on the diaphragm, the biopsy specimens from other intraperitoneal sites were positive.

The rate of survival after a second-look operation varied directly with the volume of tumor found at that operation (Fig. 1) and the amount of tumor left behind

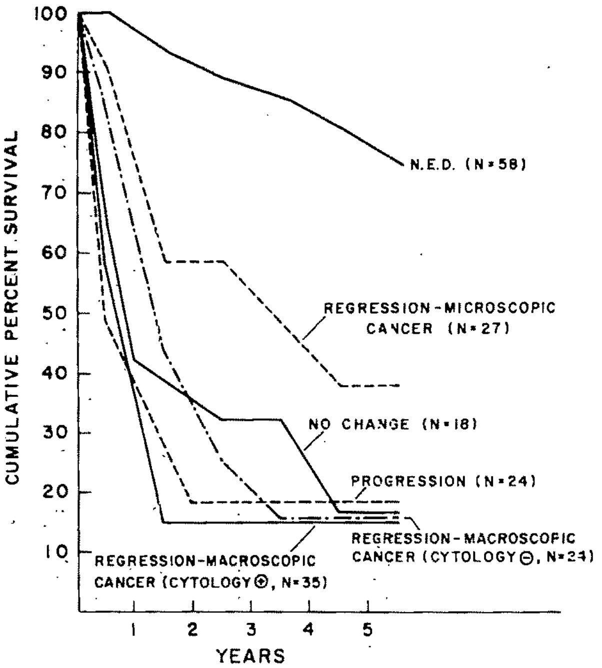

Fig. 1. Survival rate after second-look operation. The chemotherapy response was determined at the second-look operation. *N* = number of patients: *cytology* ⊖ = no malignant cells present in pelvic and paracolic space washings; *cytology* ⊕ = malignant cells present in washings.

at the second-look operation. The 5-year survival rate after second-look operations for patients with no evidence of disease was 72%, and 38% for patients with only microscopic tumor. Patients who were found to have lesions which had definitely regressed but were visible at the time of the second-look operation (2 cm in diameter—macroscopic disease) had a 5-year survival rate of 15%. Patients with no change in the status of the tumor from the time of the initial operation had a 5-year survival rate of 17%, and patients with progression of their tumor had a 5-year survival rate of 18%.

Resection of residual tumor at the second-look operation influenced the subsequent survival rate (Fig. 2). The 2-year and 5-year survival rates for patients who underwent total removal of residual tumor at the second-look operation were 47.5% and 27.0%, respectively; and if only partial removal was performed, leaving residual tumor that was less than 2 cm in diameter, the 2-year and 5-year survival rates were 29.5% for each interval. The 2-year and 5-year survival rates if residual tumor was greater than 2 cm were 9.0% for each interval. Only one patient who had residual tumor greater than 2 cm in diameter at the second-look operation had NED 60 months after the second-look procedure.

Patients treated with chemotherapy after positive second-look operations had better 2-year and 5-year survival rates than did patients who were treated with radiation therapy (Fig. 3). Patient survival rates for 2 years and 5 years with chemotherapy were 34.3% and 26.7%, respectively, whereas the corresponding survival rates with radiation therapy were 24.4% and 16.1%. This is particularly significant because only patients with residual tumor less than 2 cm in diameter at second-look operations were treated with radiation. Patients with tumors larger than 2 cm were treated with chemotherapy.

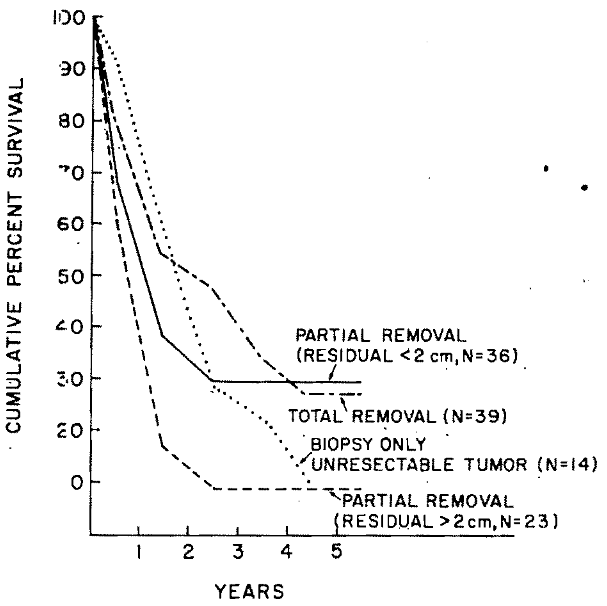

Fig. 2. Survival rate after second-look operation as determined by the maximum diameter of the largest residual tumor mass upon completion of the operation.

Significant myelosuppression after chemotherapy was observed in many patients who underwent whole-abdomen irradiation after second-look operations. Twenty-two of 49 patients treated by means of the moving-strip radiation technique, with additional irradiation of the pelvis, had their treatments interrupted or discontinued because of myelosuppression which was not necessarily related to the amount of prior chemotherapy.

Complications of second-look operations are seen in Table VI. Eighteen patients had a wound infection, 17 had an infection of the urinary tract, and 16 had hemorrhage that exceeded 500 cc.

The number of days of hospitalization is shown in Table VII. Until recently, patients were frequently kept in the hospital until a subsequent course of chemotherapy had been administered. At present, most patients are discharged on the seventh postoperative day and receive chemotherapy as outpatients.

Table VI. Complications of second-look procedure

Wound infection	18
Urinary tract infection	17
Hemorrhage > 500 cc	16
Pulmonary	10
Other medical	8
Bowel injury—postoperative	5
Bowel injury at "second-look" operation	2
Postoperative intestinal obstruction	2
Other	9
None	124

Table VII

No. of days of hospitalization	*No. of patients*
0-5	—
6-9	55
10-14	101
15-19	21
20-24	5
25-29	3
30-39	5
51	1
85	1
Total	192

The most severe long-term complication in this group of patients with the prolonged survival rate was leukemia. Five patients developed leukemia, which was diagnosed as acute granulocytic leukemia in three of them. All five patients were long-term survivors on chemotherapy (range, 43 to 184 months). One patient developed subacute leukemia (based on bone marrow findings) after receiving nine courses of melphalan and moving-strip abdominal irradiation with additional irradiation to the pelvis. The four patients with acute leukemia received 18 to 44 courses of melphalan, and three had no evidence of ovarian carcinoma at the time of their deaths. Three patients were treated with chemotherapy for acute leukemia, but no patient survived more than 3 months from the time of diagnosis.

Third-look procedures

Eight patients underwent third-look operations upon completion of a program of chemotherapy after a positive second-look operation. Four patients had NED at the time of the third-look operation. However, two of the four patients developed recurrent cancer 15 and 57 months later. The other four patients had persistent disease at the time of third-look operation, and their treatment was changed because of this finding.

Comment

The management of patients with epithelial tumors of the ovary is hampered by a lack of diagnostic tests which are highly sensitive for detecting residual or recurrent tumor after the initial operation. There are no blood tests, such as the chorionic gondadotropin titer, alpha-fetoprotein assay, or carcinoembryonic antigen level, or diagnostic radiographic techniques, including ultrasound, that have been reliable in demonstrating residual tumor or its response to treatment.[1, 2] The clinician must direct chemotherapy on the basis of findings by palpation of the abdomen, bimanual pelvic examination, chest x-ray films, laparoscopy, and second-look operations.

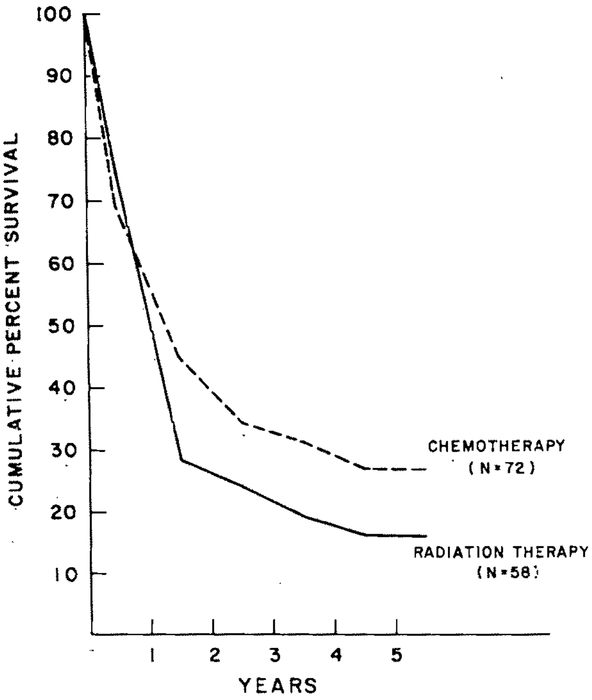

Fig. 3. Survival rate after second-look operation as influenced by subsequent therapy, radiation or chemotherapy.

The diagnostic inaccuracy of the clinical examination is demonstrated in the current series. Sixty-four of 128 patients who were found to have residual tumor at the second-look operation were thought, prior to the operation, to be free of disease by abdominal and pelvic examination. Conversely, five patients with NED at the time of a second-look operation had, prior to the second-look operation, palpable masses which were thought to be cancer.

It is now possible to state that some patients with ovarian cancer are cured by chemotherapy. Eight patients with advanced ovarian cancer had NED 5 years or more after negative second-look operations and were probably cured by chemotherapy. Ten patients with advanced ovarian carcinoma had NED 35 or more months after negative second-look operations (potential 5-year chemotherapy cures).

If we include the patients with Stage I and Stage II disease, 58 patients in the group would have been continued on chemotherapy without a second-look operation and would have been exposed unnecessarily to the possibility of developing prolonged myelosuppression or, perhaps, leukemia. Acute granulocytic leukemia developed in four of 30 patients in this series who received more than 16 courses of melphalan.

Laparoscopy has been proposed as an alternative to second-look operations.[3, 4] Twenty-four patients in the entire series had no tumor visible at the time of the second-look operation, but did have microscopic disease disclosed by random biopsies; and three patients who were given prophylactic chemotherapy were found to have microscopic disease by cytologic evaluation of the pelvis and lateral paracolic spaces. The role of laparoscopy in the management of patients suitable for a second-look operation should be to determine whether the patient has unresectable or diffuse disease. Should extensive or unresectable cancer be found at laparoscopy, confirmation of the extent of the disease by biopsy via the laparoscope is appropriate, and treatment may be continued without subjecting the patient to laparotomy. Should laparoscopy fail to demonstrate persistent disease, laparotomy and a meticulous search for tumor masses must be performed. Laparotomy is necessary because persistent disease in the pelvis or the mesentery or serosa of the small bowel, the paracolic spaces, and the para-aortic nodes and retroperitoneal area can be detected only by visual inspection and/or biopsy. If laparoscopy discloses localized residual cancer, then a laparotomy may be indicated, since patients who have all gross tumor removed by a second-look operation have an improved 2-year and 5-year survival rate (Fig. 2).

The timing of the second-look operation in relation to the number of courses of chemotherapy is extremely important. Little benefit accrues to the patient if the operation is performed as soon as an unresectable tumor becomes resectable, or if the operation is done because the patient has developed prolonged myelosuppression between courses of chemotherapy. The patients who received 12 or more cycles of chemotherapy had the best survival rates in this series. The time interval over which the total chemotherapy was administered did not influence prognosis. Seven patients treated with melphalan developed recurrent carcinoma after negative second-look operations. Five of these patients received less than 12 courses of chemotherapy.

Seven patients who had cancer at the time of the second-look operation survived 5 or more years free of disease and clearly benefited from the operation. Since the presence of persistent disease was not appreciated clinically, the second-look findings determined the subsequent management of these patients.

Patients who were found to have minimal residual tumor at second-look operations were initially treated with radiation therapy to the whole abdomen, with additional irradiation of the pelvis. Patients who were treated in this manner had a poorer survival rate (Fig. 3) than did patients with more extensive cancer who were treated with chemotherapy. The current policy is to treat all patients with chemotherapy if cancer is found at a second-look operation.

Third-look procedures have been performed on eight patients and appear to have a role in the management of patients after completion of a second program of chemotherapy.

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Progestogen-dependent endometrial protein in women: Tissue concentration in relation to developmental stage and to serum hormone levels

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The presence of a progestogen-dependent endometrial protein (PEP) in women was previously described. The present study was undertaken to determine whether the occurrence or the concentration of PEP in endometria is related to the developmental stage of tissues and/or to the levels of endogenous progesterone (P) or 17β-estradiol (E) to which the tissues are exposed. Samples of serum and endometrial tissue in different developmental stages were obtained from pregnant women and women with normal menstrual cycles and were analyzed for PEP. In addition, the sera were analyzed for P and E. The occurrence of PEP in the endometrium of all of the women who were studied was found to be correlated with the heightened secretory activity and with the decidual change in the tissue. Furthermore, in women with normal cycles, the detection of PEP was associated with high serum levels of P. Comparison of serum levels of P with endometrial levels of PEP in pregnant women and in women in the mid-secretory phase of the menstrual cycle indicated that a 2.8-fold rise in serum levels of P during pregnancy was accompanied by a comparable (about threefold) rise in tissue levels of PEP. Therefore, we conclude that, in pregnant women and women with normal cycles, the levels of PEP in the endometrium reflect developmental changes within the endometrium, and that those levels are directly related to serum levels of P. (AM. J. OBSTET. GYNECOL. 138:1131, 1980.)

BOTH NATURAL and synthetic progestogens are being used in the clinics for treatment of luteal-phase insufficiency, habitual and threatened abortions, endometrial hyperplasia, and as adjuvant therapy in endometrial and breast cancers. In addition, clinical trials are under way in many developing nations which use synthetic progestogens as a means of clinical contraception in the form of oral pills, subdermal silicone rubber implants, and intrauterine and intravaginal devices. In spite of such widespread use of progestogens, very little is known about their specific biochemical effects on human target tissues. At the Conference of Biochemical Action of Progestin sponsored by the New York Academy of Sciences, in 1976, we presented evidence which indicated that the human uterine decidua of pregnancy synthesizes a specific antigenic protein.[1] In endometria from women with normal cycles, this protein was detected in about 47% of the luteal phase specimens, but in none of the follicular-phase specimens, thereby suggesting that the antigen was a progestogen-dependent endometrial protein (PEP). Basic studies related to PEP have been published elsewhere.[2, 3] The present study was undertaken to determine whether the concentration of PEP in the human endometrium is related to the developmental changes

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This work was supported by the Ford Foundation (Grant No. 680-0900) and the National Institute of Child Health and Human Development (Grant No. HD-090622).

Received for publication April 2, 1979.

Revised July 15, 1980.

Accepted July 21, 1980.

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Table I. Serum levels of P and E in women with normal cycles and in women 5 weeks' to 10 weeks' pregnant

Endometrial status of donor*	No. of samples	Hormone levels	
		P (ng/ml) Mean ± SE†	*E (pg/ml) Mean ± SE†*
Proliferative phase	20	1.42 ± 0.18	147 ± 22
Early secretory phase	8	3.24 ± 0.55	165 ± 24
Midsecretory phase	18	9.20 ± 1.50	231 ± 39
Early pregnancy (5-10 weeks)	27	25.58 ± 2.82	1,202 ± 196

*Determined by histologic examination of hematoxylin and eosin-stained sections of endometrium.

†SE = standard error.

within that tissue and/or to levels of endogenous progesterone (P) or estradiol (E) to which that particular tissue has been exposed.

Material and methods

Human material. All specimens used in this study were obtained with the informed consent of the patients who were treated at the Albany Medical Center Hospital, Albany, New York. Samples of endometrium were procured from 56 nonpregnant women, aged 20 to 45 years, who underwent curettage or hysterectomy for pelvic disease, or who underwent both tubal ligations and curettage for sterilization. In addition, samples of uterine decidua were obtained from 27 women who elected to terminate early pregnancy (5 to 12 weeks) by menstrual induction. Besides specimens of tissue, samples of blood were collected along with the tissue from 46 nonpregnant and 27 pregnant women.

A portion of each tissue obtained from women with normal menstrual cycles was promptly fixed in buffered formalin for routine histologic examination, and the remaining tissue was used for immunologic studies. The formalin-fixed tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin by standard procedures. Sections of endometrial tissue were examined by light microscopy and broadly classified as proliferative, early secretory, midsecretory, or late secretory phase, according to the guidelines described by Noyes and associates.[4]

Uterine decidua were unavoidably contaminated with blood and fetal elements. To minimize these contaminants, the tissues were processed as described elsewhere.[2, 3] Portions of each tissue were used for histologic examination and for immunologic studies. The histologic examination of the processed decidua showed that they were comprised mainly of decidual cells, hypersecretory glands, and a few placental villi.

Tissue cytosols were generated by homogenizing the tissues in cold (4° C) 0.05M Tris HCl buffer, pH 7.4, followed by centrifugation of the homogenates at high speed (110,000 × g, 90 minutes). The clear supernatants or cytosols were aspirated and stored in aliquots at −20° C until used for quantitation of proteins and PEP. The samples of blood were clotted and centrifuged to obtain sera. The sera were stored at −20° C until used for the detection of PEP and quantitation of E and P.

Analysis of PEP. PEP was detected by Ouchterlony's double immunodiffusion test and by rocket immunoelectrophoresis with the use of antisera produced in rabbits against a preparation of PEP which was partially purified by concanavalin A–Sepharose chromatography and polyacrylamide gel electrophoresis (PAGE). As indicated elsewhere,[3] analysis of this PEP preparation by PAGE revealed one major protein band containing the PEP and 10 minor protein bands devoid of PEP. Immunodiffusion tests demonstrated the presence of transferrin, alpha-1 antitrypsin, and ceruloplasmin in the partially purified PEP preparation.[3] Before use, the antisera were absorbed with quality controlled male serum. The absorbed antiserum used in the present study reacted with the cytosols of the decidua of pregnancy or of secretory endometria of women with normal cycles, with the formation of a single diffuse precipitin line corresponding to PEP. The antiserum did not react with adult male or female sera. The details of the procedures for purification of PEP, immunization of rabbits against PEP, and detection of PEP by double immunodiffusion test and rocket immunoelectrophoresis have been described elsewhere.[2, 3, 5] The procedure of rocket immunoelectrophoresis was also used to determine tissue concentrations of PEP. Briefly, glass plates (8.4 by 9.4 cm) were coated with 1% agarose in barbital buffer (pH 8.6, ionic strength 0.02) containing the immunoglobulin fraction (0.57 mg per milliliter of agarose) of the absorbed antiserum. The immunoglobulin fraction was prepared by the addition of solid ammonium sulfate to the absorbed antiserum to achieve 40% saturation. A set of 14 antigen wells (4 to 5 μl capacity) was punched in the agarose bed with an LKB well puncher (LKB Instruments, Hicksville, New York) and filled with test antigen solutions or graded amounts (20 to 320 ng) of reference antigen. The reference antigen was partially purified from the decidual cytosol, as described previously.[3] The samples were electrophoresed for 4 hours at 8 V/cm at 4 to 7° C with the use of barbital buffer as the tank buffer and the LKB 2117 Multiphore and the LKB 2103 Power Supply (LKB Instruments). After electrophoresis, the immunodiffusion plates were washed with saline solution,

Table II. Precision and sensitivity of standard curve for PEP

PEP (ng)	Evaluation based on rocket height			Precision and sensitivity (Δ h) (ng)
	Mean	*SD**	*CV† (%)*	
20	2.5	0.45	17.9	4.9
40	4.0	0.41	10.2	4.5
80	8.3	0.48	5.8	5.3
160	15.9	0.25	1.6	2.7
240	22.3	0.65	2.9	7.1
320	27.5	0.41	1.5	4.5

*SD = standard deviation.
†CV = coefficient of variation.

and the immunoprecipitates were stained with Coomassie Blue. A dose-response curve was generated by plotting the height of the rocket against the concentration of reference antigen, and sample values were interpolated from a straight-line dose response curve. The tissue concentration was expressed in terms of units per milligram of cytosol protein, one unit being equivalent to the total response obtained by 1 μg of reference PEP. The colorimetric method of Lowry and associates[6] was used to determine total proteins.

Assay of serum E and P. Serum concentrations of E and P were measured by the conventional radioimmunoassays with the use of highly specific antisera against E or P. Such antisera were produced in rabbits, using 17α-progesterone hemiglutaraldehyde–bovine serum albumin and 17β-estradiol-3-0-carboxymethyl–bovine serum albumin as specific antigens for the production of anti-P and anti-E, respectively. These antisera were fully characterized[7, 8] and supplied to us by Dr. P. N., Rao, of the Southwest Foundation for Research and Education, San Antonio, Texas.

The statistical significance of the differences in the concentrations of PEP, E, or P was evaluated by Student's t test, and corrections were made for unequal group size. A value of P smaller than 0.05 was considered to be significant.

Results

On the basis of histologic examination, the 56 tissue samples obtained from nonpregnant women were classified as proliferative (N = 27), early secretory phase (N = 11), and midsecretory phase (N = 18). None of the 56 tissues was in the late secretory phase.

The samples of serum were also designated as proliferative, early secretory or midsecretory phase, after histologic classification of the donor endometria. These samples were analyzed for E and P by radioimmunoassays.

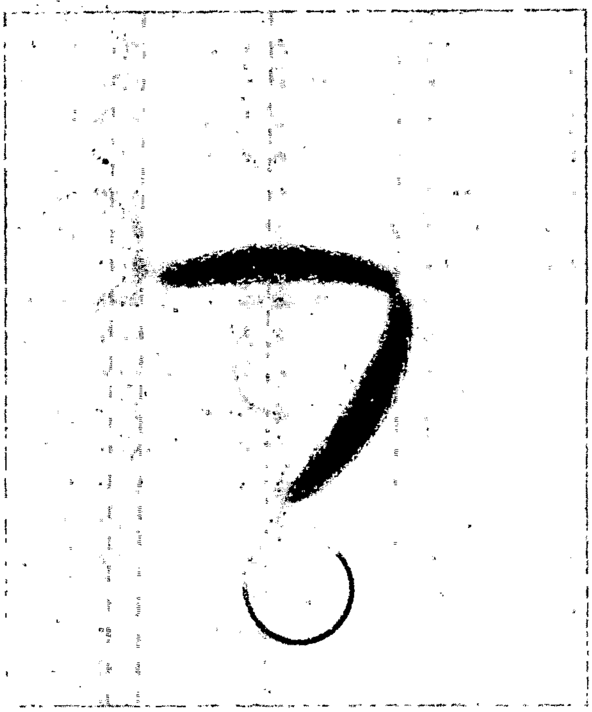

Fig. 1. Detection of PEP by Ouchterlony's double immunodiffusion test. The peripheral antigen wells (clockwise from top) contain cytosol of the uterine decidua of pregnancy and cytosol of the endometrium of the early secretory phase, the midsecretory phase, and the proliferative phase, and nonpregnancy serum and pregnancy serum. The center well contains anti-PEP.

The results of hormone analysis, shown in Table I, demonstrate that the levels of P in the midsecretory phase (mean ± SE = 9.20 ± 1.50 ng/ml) were significantly higher than those in the early secretory phase (3.24 ± 0.55 ng/ml) or proliferative phase (1.42 ± 0.18 ng/ml). However, the mean concentrations of E in these three groups of sera did not differ significantly. In the pregnancy sera, the levels of both P (25.58 ± 2.82 ng/ml) and E (1,202 ± 196 pg/ml) were significantly higher than the levels in the sera of women with normal cycles.

Samples of sera and tissue cytosols were screened for the presence of PEP by Ouchterlony's double-immunodiffusion test. The results are shown in Fig. 1. As for the tissue cytosols, the presence of PEP was demonstrated in all 27 prepared from uterine decidua and in 18 prepared from midsecretory phase endometria of women with normal cycles. In contrast, PEP could not be detected in cytosols of 27 endometria of the proliferative phase or 11 endometria of the early secretory phase. Also, PEP could not be detected in any of the nonpregnancy or pregnancy sera.

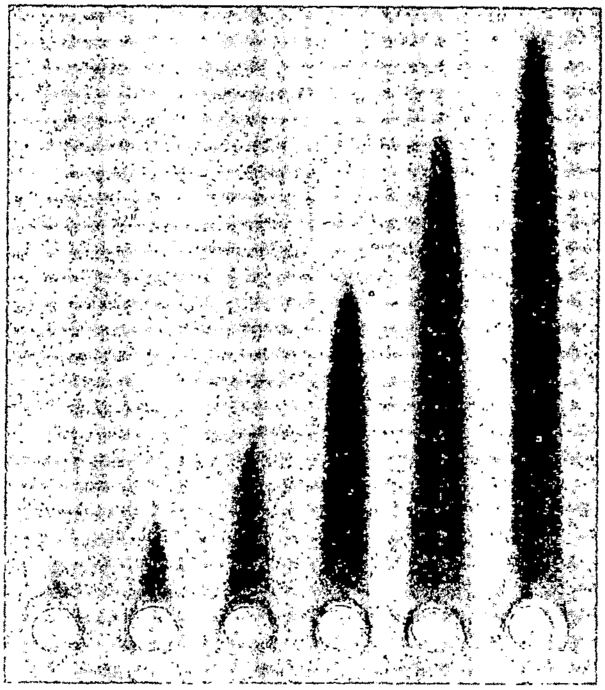

Fig. 2. Quantitation of PEP by rocket immunoelectrophoresis. Dilutions of partially purified PEP electrophoresed in agarose gel containing immunoglobulin fraction of the anti-PEP antiserum. Immunoprecipitates stained with Coomassie Blue.

Table III. Tissue levels of PEP in endometria of women with normal cycles and pregnant women

Endometrial status of donor	*N*	*No. of specimens containing antigen*	*Tissue concentration of PEP (U/mg cytosol protein; (mean ± SE)*
Proliferative phase	27	0	ND
Early secretory phase	11	1	1
Midsecretory phase	18	18	54 ± 8
Early pregnancy (5 to 10 weeks)	27	27	163 ± 41

ND = Not detected.

One unit = 1 μg of partially purified antigen.

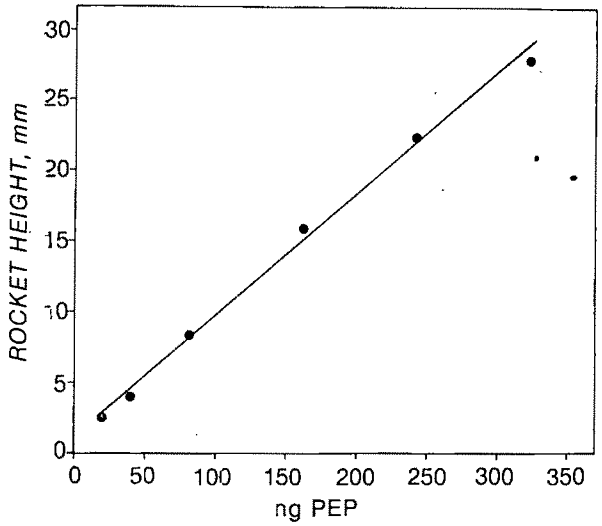

Fig. 3. Quantitation of PEP by rocket immunoelectrophoresis. Relationship between the peak heights and the amount of protein shown in Fig. 2.

In subsequent studies, attempts were made to determine whether, in the individual women with normal cycles or pregnant women, the tissue levels of PEP bore any relationship to serum levels of P or E. A rocket immunoelectrophoretic technique was developed for quantitation of tissue levels of PEP. A standard curve was obtained with the use of quadruplicate aliquots that contained 20 to 320 ng of PEP (Table II and Figs. 2 and 3). Sensitivity and precision were evaluated as suggested by Ekins and Newman[9] for each standard point of the curve. Although the data for precision would suggest sensitivity of 4.9 ng per assay aliquot, the coefficient of variation at this level was too high (17.9%). For practical purposes, the sensitivity was 40 ng per assay aliquot, with a coefficient of variation of 10.2%. The precision over the range of 40 to 320 ng varied from 2.7 to 7.1 ng. Within this range, the intra-assay coefficient of variation was from 1.5% to 10.2%, and the interassay coefficient of variation was from 7.7% to 15%. Analysis of samples of a decidual cytosol prepared by serial dilution of the cytosol with the assay buffer generated a curve which was parallel to the standard curve (results not shown).

The assay was specific for PEP, since several known pregnancy proteins, including transferrin, ceruloplasmin, alpha-1 antitrypsin, ferritin, blastokinin, and human chorionic gonadotropin, did not react with the anti-PEP. PEP could not be detected in samples of serum by rocket immunoelectrophoresis.

The results of analyses of tissue levels of PEP are shown in Table III. PEP could not be detected in the cytosols of 27 tissues of the proliferative phase nor 10 of the 11 tissues of the early secretory phase. In the cytosol of one tissue of the early secretory phase, the concentration of PEP was very low (1 U/mg of cytosol protein). PEP was detected in all 18 endometria of the midsecretory phase and in all 27 uterine decidua of early pregnancy. Moreover, the levels of PEP in the uterine decidua (mean ± SE = 163 ± 41 U/mg of cytosol protein) were significantly higher than those in the endometria of the midsecretory phase (54 ± 8 U/mg of cytosol protein). Comparison of serum levels of P (Table I) and endometrial levels of PEP (Table III) in groups of pregnant women and women in the midsecretory phase of the menstrual cycle indicated that a 2.8-fold increase in serum levels of P during pregnancy was accompanied by a comparable (threefold) increase in tissue levels of PEP.

The relationship between the detection (or nondetection) of PEP and serum levels of E and P in individual women with normal cycles is illustrated in Fig. 4. The frequency of occurrence of PEP in the endometrium was found to be very high (15 of 18, or 83%) when the serum level of P in the tissue donor was 5 ng/ml or greater. In the majority of women with normal cycles whose serum level of P was greater than 5 ng/ml and whose endometria contained PEP, the serum level of E was greater than 100 pg/ml. However, no other quantitative relationship was apparent between serum levels of E and the detection of PEP in the endometria in the individual women with normal menstrual cycles.

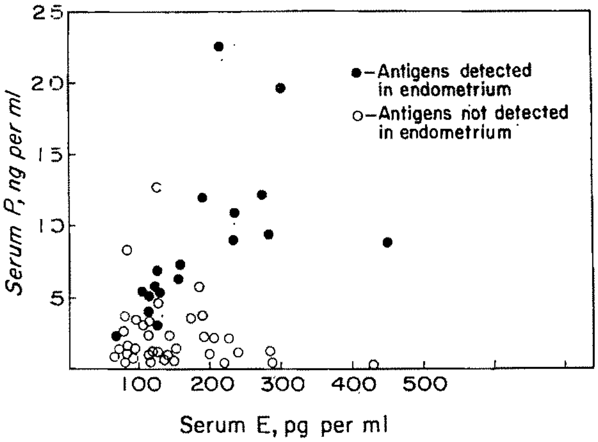

Fig. 4. Relationship between the occurrence of PEP and serum levels of E and P in individual women with normal menstrual cycles.

Comment

The findings of this study clearly demonstrate that a direct relationship exists between the tissue level of PEP in the endometrium and the serum level of P in pregnant women and in women with normal menstrual cycles. The occurrence of PEP in the endometria of women with normal cycles was found to be related to high concentrations of serum P, but not of E, thereby suggesting that the endometrial synthesis of PEP is regulated mainly by progesterone and not by estrogens. This suggestion is further strengthened by our recent observation that medroxyprogesterone acetate stimulates in vitro synthesis of PEP in explants of proliferative-phase endometria (unpublished observations). Undoubtedly, estrogens may facilitate the synthesis of PEP in the endometrium by maintaining high levels of progesterone receptors in the target tissue.[10] The present study, as well as the study previously reported by Moghissi and associates,[11] clearly demonstrates that progesterone can be detected in both the peripheral blood and endometrial tissue of women with normal cycles even during the preovulatory phase. We previously demonstrated that the endometria of the late proliferative phase incorporate small amounts of radioleucine into PEP.[2] Therefore, it is apparent that progesterone promotes the synthesis of PEP in the endometrium even during the preovulatory phase of the menstrual cycle, but in amounts too small to be detected by the relatively insensitive techniques of immunodiffusion. The present study demonstrates that during the menstrual cycle the level of PEP in the endometrium rises very rapidly shortly after the tissue has attained the early secretory developmental phase, and it continues to rise during early pregnancy. The endometrial cell type(s) involved in the synthesis of PEP has(have) not yet been identified. However, the detection of PEP in the tissues during the midsecretory phase of the menstrual cycle and during early pregnancy when the secretory activity of the glands is at its peak suggests that PEP is synthesized and secreted by the endometrial glands. Recently, we detected the presence of PEP in human amniotic fluid, thus indicating again that PEP may be secreted by the endometrium and transported to the amniotic sac.

Very few progestogen-dependent proteins have been identified in the human endometrium. Tseng and Gurpide[12] have shown that the activity of estradiol dehydrogenase in the human endometrium is regulated by progesterone. Feigelson[13] demonstrated a blastokinin-like protein in the luteal phase uterine washings of women. In contrast, Voss and Beato[14] did not detect blastokinin in human uterine secretions. Shapiro and Forbes[15] have reported that the synthesis of a specific endometrial protein is stimulated by the exposure of proliferative phase endometria to progestogens in vivo and in vitro. Sutcliffe and associates[16] have described the presence of an alpha uterine protein (AUP) in human amniotic fluid and offered evidence that this protein is also to be found in the decidua. Our recent studies[5] strongly suggested that the PEP being investigated by us is related to the AUP described by Sutcliffe and associates.[16]

Recently, we developed a radioimmunoassay for PEP and detected and quantitated PEP in samples of peripheral blood from women in early pregnancy.* Quantitation of a hormone-dependent protein such as PEP may offer clinicians a means to monitor endometrial development in high-risk pregnancies.

We wish to extend our appreciation to Dr. H. Hussain, Department of Pathology, Albany Medical College, for careful histologic analysis of the endometria used in the present study, and to thank Professor Herbert I. Jacobson for his constructive comments on the manuscript.

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*Unpublished observations.

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Plasma estriol and its conjugates following oral and vaginal administration of estriol to postmenopausal women: Correlations with gonadotropin levels

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A study was designed to compare the metabolic fate and the biologic effects of 4 mg of estriol (E_3) administered either orally or vaginally to six postmenopausal women. Blood samples were collected every hour for 6 hours and five different estriol fractions as well as gonadotropins were measured. Vaginal E_3 administration resulted in a decline of 45% in luteinizing hormone (LH) levels and 17% in follicle-stimulating hormone (FSH) levels at 6 hours after treatment ($p < 0.05$). In contrast, the administration of 4 mg of E_3 orally did not produce a decline of LH and FSH, despite the fact that the serum levels of E_3-3-sulfate, E_3-3-sulfate-16-glucosiduronate, estriol-3-glucosiduronate, and estriol-16-glucosiduronate were all fourfold to 24-fold higher after oral administration than after vaginal estriol administration. However, since the levels of unconjugated E_3 were higher after the vaginal than after the oral administration of estriol, we conclude that only unconjugated E_3 suppresses gonadotropins. (AM. J. OBSTET. GYNECOL. 138:1137, 1980.)

IN AN EARLIER REPORT from one of our laboratories, we have shown that estrogens administered vaginally are absorbed systemically and are biologically active.[1] We subsequently extended these studies and found that 0.5 mg of estriol (E_3) inserted vaginally in postmenopausal women caused the same minimal decrease in the concentration of serum luteinizing hormone (LH) as 8 mg given orally.[2] Therefore, it can be predicted that administration of 4 mg of E_3 orally will have little or no effect on LH values while the administration of an equivalent dose vaginally will have a dramatic effect. To determine why the route of E_3 application was critical for determining its biologic effect, we quantified and identified the various forms of E_3 found in the serum after the oral and vaginal administration of 4 mg of E_3.

Subjects and methods

Six hypogonadal women visiting the menopause clinic, after signing informed consent, volunteered to participate in this study. Two women (Numbers 3 and 5), aged 50 and 65, were postmenopausal and four (aged 35, 51, 62, and 64) had previously undergone oophorectomy for benign gynecologic diseases. None had received any drugs or hormones for at least 2 months prior to entering the study and all had vasomotor symptoms.

Each patient was studied twice, once after receiving 4 mg of E_3 orally and again, 4 weeks later, after having 4 mg of E_3 dispensed in 2 ml of saline, placed in the vagina. Each study was always begun at 8:00 to 9:00 AM

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Supported by Grant CA 02071 from the National Cancer Institute.

A preliminary report was presented at the Twenty-seventh Annual Meeting of the Society for Gynecologic Investigation, Denver, Colorado, March 19-22, 1980.

Received for publication March 18, 1980.

Revised and accepted July 31, 1980.

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0002-9378/80/251137+05$00.50/0 © 1980 The C. V. Mosby Co.

Table I. Concentration of FSH in serum of subjects following oral (O) and vaginal (V) administration of E_3

Hour	*Subject 1*		*Subject 2*		*Subject 3*		*Subject 4*		*Subject 5*		*Subject 6*		*Mean ± SE*	
	O	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*
Baseline	112	104	166	>200	>200	>200	122	184	44	72	88	88	120.3 ± 22.4	141.3 ± 24.3
1	−11	+8	−18	0	−36	−18	+80	−11	+9	−5	−36	−16	−2.2 ± 17.6	−7.2 ± 4.0
2	−18	+2	−13	+16	−28	−22	+80	−7	−2	−20	−46	+11	−4.8 ± 17.7	−3.2 ± 6.4
3	−30	−40	−1	−22	−18	0	+80	−17	−2	−3	−36	−16	−1.6 ± 17.0	−17.4 ± 5.3*
4	−41	−12	−18	−28	−22	−18	+80	−22	−16	−20	−36	−16	−9.1 ± 18.0	−19.2 ± 2.3*
5	−25	−8	−13	−28	−30	−24	+80	−11	+9	0	−11	−16	1.3 ± 16.4	−14.4 ± 4.2*
6	0	−17	−11	−32	−12	−12	+80	0	−9	−8	−30	−11	2.8 ± 15.6	−13.5 ± 4.4*

Values are in milli–International Units per milliliter for baseline followed by percent change from baseline for 6 succeeding hours.

*$P < 0.05$ (significant as compared to baseline values).

Table II. Concentration of LH in serum of subjects following oral (O) and vaginal (V) administration of E_3

Hour	*Subject 1*		*Subject 2*		*Subject 3*		*Subject 4*		*Subject 5*		*Subject 6*		*Mean ± SE*	
	O	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*	*O*	*V*
Baseline	29	36	60	84	41	96	70	88	47	50	26	27	45.5 ± 7.0	63.5 ± 12.0
1	−14	−17	−70	−24	+80	−24	0	−1	−38	−4	−12	+4	−9.0 ± 20.4	−14.8 ± 4.9
2	+3	−39	−26	−52	+71	−21	+3	−8	−16	−16	−23	−23	2.0 ± 22.2	−26.5 ± 6.6*
3	−17	−42	−32	−56	+100	−41	+3	−31	−40	−44	−45	−38	5.5 ± 22.2	−42.0 ± 3.4*
4	+66	−58	−37	−38	+70	−42	−25	−42	−32	−44	−46	−19	−0.3 ± 21.8	−40.5 ± 5.1*
5	+34	−39	−25	−31	+107	−41	−15	−52	−40	−46	−23	−36	11.0 ± 22.2	−40.8 ± 3.0*
6	+86	−66	−30	−48	+114	−62	−28	−30	−38	−46	−62	−38	16.8 ± 29.0	−48.3 ± 5.6*

Values are in milli–International Units per milliliter for baseline followed by percent change from baseline for 6 succeeding hours.

*$P < 0.05$.

after overnight fasting. Samples for determination of baseline blood levels were drawn through an intravenous heparinized catheter twice before the drug administration and again every hour for 6 hours. Serum was separated and stored at −20° C until analyzed.

LH and follicle-stimulating hormone (FSH) were measured by radioimmunoassay.[3] The various E_3 fractions were measured as follows.

For unconjugated E_3 2 ml of serum was extracted twice with 3 ml of ether. ^3H-E_3 (500 cpm) was added to the extracts, which were then evaporated. The extracts were dissolved in 3 ml of water and extracted first with benzene-hexane (2 × 3 ml), then with ether (2 × 3 ml). The ether was evaporated, dissolved in benzene-methanol (85-15), and transferred to a column (10 by 0.7 cm) of Sephadex LH-20 suspended in the same solvent.[4] The E_3 was eluted with benzene-methanol (85-15) in the 4 to 6 ml cut. The solvent was evaporated, 1 ml of ethanol was added to the samples, and 0.2 ml was taken for estimation of recovery. Appropriate aliquots, usually 0.2 ml, were submitted to radioimmunoassay as described.[5]

The residual serum which remained following the extraction of the unconjugated estriol with ether, was assayed for the four major conjugates of estriol, namely, estriol-3-sulfate-16-glucosiduronate* (E_3-SG),[1] estriol-3-sulfate† (E_3-3S), estriol-16-glucosiduronate (E_3-16G), and estriol-3-glucosiduronate‡ (E_3-3G) as previously described.[6] The aliquots submitted to radioimmunoassay were adjusted so that the counts per minute fell on the appropriate portion of the standard curve. The coefficient of variation of these methods was less than 12%.

Statistical analysis. The FSH and LH levels measured at each hour after the E_3 administration were compared to those before treatment (baseline) and the percent change from baseline was calculated. The mean levels during the oral and vaginal routes were compared by means of the paired t test.[7]

Results

The effects of oral and vaginal E_3 administration on serum FSH and LH levels are shown respectively in Tables I and II and in Fig. 1. The vaginal administration of 4 mg of E_3 was associated 3 to 6 hours later with a fall of 13.5% ± 4.4% (SEM) to 19% ± 2.3% (SEM)

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*17β-Hydroxyestra-1,3,5(10)-trien-3-*yl*-sulfate-16α-yl-β-D-glucopyranosiduronate.

†16α,17β-Dihydroxyestra-1,3,5(10)-trien-3-*yl*-sulfate.

‡16α,17β-Dihydroxyestra-1,3,5(10)-trien-3-*yl*-β-D-glucosiduronate.

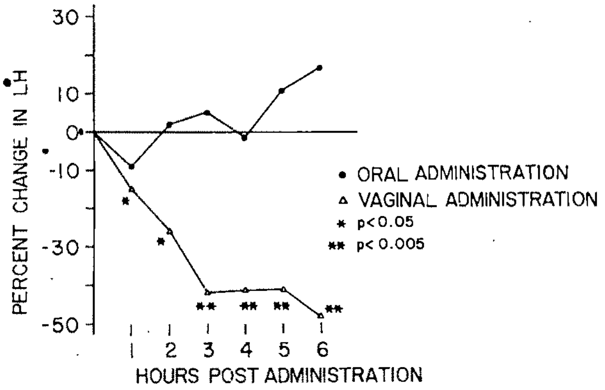

Fig. 1. The percent change in serum LH concentrations over 6 hours following the oral and vaginal administration of 4 mg of E_3.

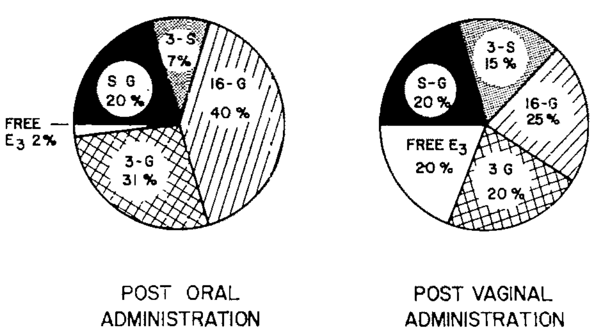

Fig. 2. The distribution in serum of the various conjugates of E_3 as well as unconjugated E_3 (free) after the administration of 4 mg of oral or vaginal E_3. The mean total E_3 concentration during the 6 hours after its administration was considered as 100%, and the mean percentages of unconjugated (free) E_3 and of E_3-3G, E_3-16G, E_3-3S, and E_3-SG are depicted.

($p < 0.05$) in FSH levels and 40.5% ± 5.1% (SEM) to 48.3% ± 5.6% (SEM) ($p < 0.05$) in LH levels. In contrast, the oral E_3 administration had no effect on FSH and LH levels ($p > 0.05$).

To elucidate the reasons for the differences in biologic activity exerted by the vaginal and oral E_3, we have studied the metabolic fate of the administered E_3 in detail. Data on the concentrations of four different E_3 conjugates as well as unconjugated E_3 of one representative patient are presented in Table III. Data on the conjugates of the other five patients were very similar to those of this patient and are not presented. In the oral phase of the study, there was a prompt increase in the concentrations of all four conjugates in the blood. Throughout the 6-hour period E_3-16G and E_3-3G accounted for greater than two thirds of the total E_3 measured. However, a decline in the concentrations of E_3-16G and a reciprocal increase in those of E_3-3G resulted in a predominance of E_3-3G at the later period. The concentration of E_3-SG and E_3-3S also declined in the latter 2 hours of the study, whereas the concentrations of unconjugated E_3 remained extremely low, never exceeding 0.031 ng/ml throughout the 6-hour study period.

The serum E_3 profile after E_3 vaginal administration was quite different. The total concentration of E_3 in serum after its vaginal administration was only about one tenth that observed after its oral administration ($p < 0.05$). The percent contribution of each form of E_3 to the total remained fairly constant throughout the 6-hour period, with E_3-16G predominating (Fig. 2). However, the most striking differences were found in the concentrations of serum unconjugated (free) E_3, which were much higher after vaginal than after oral E_3 treatment.

Since the important differences in the two modes of administration of estriol appear to be in the concentrations of unconjugated E_3, these data are presented in detail in Table IV. Within 1 to 2 hours after vaginal E_3 administration, serum unconjugated E_3 constituted approximately 30% of the total circulating E_3 and its contribution decreased to 15% to 17% in the next 4 hours. In contrast, in three of the patients, during the first 5 hours after oral E_3 treatment, the unconjugated E_3 concentration did not exceed 1% of the total circulating E_3 and its concentration amounted to less than 10% of that measured after vaginal E_3 treatment. Patients 5 and 6 did have elevations of unconjugated estriol at the sixth hour after treatment and this was reflected by a fall in LH.

Comment

The concept of administering E_3 to patients requiring estrogen replacement stems from two lines of investigation. First, experiments with rats suggest that E_3 is a weak estrogen or even an estrogen antagonist.[8, 9] Second, epidemiologic studies based on assays of urinary E_3 suggest that E_3 may be protective against breast cancer.[10] On close scrutiny, conclusions based on both lines of investigation may be flawed. For example, in the rat chronically treated with E_3, specific estrogen effects normally elicited by estradiol are observed.[11] Moreover, studies have shown that E_3 can stimulate the growth of breast tumor cells maintained in culture.[12] In the light of these apparent contradictions, we feel that further studies on the effects of E_3 should be undertaken.

In this study, we have confirmed that E_3 possesses biologic activity in women. This is apparent from the prompt suppression of LH that followed vaginal E_3 administration. The corresponding decrease in FSH was less impressive, but these differences may be attributed in part to the incidental increase in FSH levels

Table III. Concentrations of different forms of E_3 in serum following the oral and vaginal administration of E_3 in Subject 1

Route of administration	Hour	E_3-SG*		E_3-3S		E_3-16G		E_3-3G		E_3	
		ng/ml	%†	ng/ml	%	ng/ml	%	ng/ml	%	ng/ml	%
Oral	0‡	0.057	26	0.0	0	0.103	47	0.061	28	<0.01	0.00
	1	2.7	18	1.8	12	6.0	41	4.2	29	0.031	0.1
	2	6.7	27	1.6	7	11.8	48	4.6	19	0.006	0.1
	3	2.4	19	1.7	13	5.4	42	3.4	26	0.00	0
	4	1.3	14	1.3	14	3.5	38	3.1	34	0.005	0.1
	5	1.4	9	1.7	11	5.9	38	6.4	42	0.014	0.1
	6	1.8	10	1.1	6	4.9	28	9.5	55	0.015	0.1
Vaginal	0‡	0.22	81	0.00	0	0.025	9	0.027	10	<0.010	<50
	1	0.24	14	0.29	17	0.61	35	0.17	10	0.46	26
	2	0.25	14	0.40	22	0.53	30	0.19	11	0.42	24
	3	0.22	16	0.38	24	0.43	32	0.16	12	0.23	16
	4	0.16	14	0.26	22	0.38	33	0.15	13	0.21	18
	5	0.22	16	0.32	23	0.44	32	0.21	15	0.21	15
	6	0.17	13	0.29	21	0.52	38	0.18	13	0.20	15

*Abbreviations of E_3 conjugates are in text.

†Percent of total circulating E_3.

‡Baseline concentrations just prior to administration of 4 mg of E_3.

Table IV. Concentrations of unconjugated E_3 in serum following the oral and vaginal administration of E_3 in five subjects

Route of administration	Hour	Subject 2		Subject 3		Subject 4		Subject 5		Subject 6		Mean ± SE	
		pg/ml	%*	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%	pg/ml	%
Oral	0†	0	<0.1	19	22	0	<0.1	0	<0.1	11	6.5	5.0 ± 3.3	4.8 ± 3.6
	1	19	<0.1	29	<0.1	30	<0.1	6	<0.1	98	0.3	35.5 ± 13.1	0.1 ± 0.03
	2	7	<0.1	6	<0.1	0	<0.1	5	<0.1	95	0.2	19.8 ± 15.1	0.1 ± 0.01
	3	8	<0.1	9	<0.1	16	<0.1	6	<0.1	58	0.2	16.2 ± 8.6	0.1 ± 0.01
	4	17	<0.1	19	<0.1	19	<0.1	2	<0.1	140	0.7	33.7 ± 21.5	0.2 ± 0.1
	5	9	<0.1	56	<0.1	52	<0.1	124	0.5	373	3.2	104.7 ± 56.0	0.7 ± 0.5
	6	0	<0.1	10	<0.1	21	<0.1	113	0.4	1,860	10.8	336.5 ± 305.2	1.9 ± 1.7
Vaginal	0†	0		8	1.6	0		0		0		3.0 ± 1.9	0.3 ± 0.2
	1	143	17	839	27	248	51	263	11	921	52	47.9 ± 133.9	30.6 ± 7.0
	2	260	19	806	20	430	40	474	14	1,730	58	686.6 ± 221.1	29.2 ± 6.8
	3	323	19	534	13	668	40	234	8	322	21	385.2 ± 72.3	19.5 ± 4.5
	4	292	20	546	18	391	28	193	7	230	15	310.8 ± 35.6	17.7 ± 2.7
	5	206	13	483	17	320	24	317	12	215	17	291.8 ± 43.9	16.3 ± 1.7
	6	173	13	555	20	215	19	242	8	173	13	259.7 ± 60.0	14.6 ± 1.8

The mean represents the unconjugated E_3 for all six patients.

*Contribution to the calculated total E_3. The data for individual conjugates are not shown since they were similar to those those in Table III.

†Baseline concentrations just prior to administration of 4 mg of E_3.

observed in one patient (No. 4) as well as to the longer half life of FSH (3 hours) compared to that of LH (30 minutes).[13] Thus, studies of longer duration would be required to fully assess the influence of E_3 on serum FSH in postmenopausal women.

The acute administration of vaginal but not oral E_3 elicited gonadotropin suppression, and this difference may be attributed to the different routes of E_3 transport and metabolism. While the absorption of E_3 was rapid after oral or vaginal E_3 treatment, the total plasma concentration of E_3 in blood was higher after oral than after vaginal E_3 administration (15 to 64 versus 1.0 to 3.0 ng/ml). This may be only partially explained by possible loss of some of the material from the vagina. Moreover, after oral E_3 administration, the main circulating E_3 conjugates were E_3-16G and E_3-3G. Since the intestines are particularly rich in 3- and 16-glucuronyl transferase activities, it appears that the bulk of ingested E_3 was converted to the biological inactive estriol glucosiduronates prior to transport to the blood.[14] The findings of only minute amounts of unconjugated and presumably biologically active E_3 after its oral administration may explain the lack of effect on gonadotropins. A notable exception was Subject No. 6. The level of unconjugated E_3 in this patient rose after ingestion of E_3 and at 6 hours after its administration the level reached 1,860 pg/ml, explaining the suppression of the serum LH and FSH concentration. Her medical history included no abnormality that could explain this unusual profile.

In contrast, the vaginal administration of E_3 resulted in levels of unconjugated E_3 which were comparable to those of the various E_3 conjugates (Fig. 2). Thus, vaginal insertion of E_3 permits the absorption of the presumably active form of the hormone into the blood for transport to target tissues prior to its inactivation via conjugation (at the 3 and 16 positions) by enzymes residing in the enterohepatic system.[14] It should be noted that because the metabolic clearance rate from blood of glucoconjugates is much faster than that of sulfoconjugates, the plasma concentrations of the latter in blood were disproportionately higher.[15] In all likelihood, only small amounts of the absorbed E_3 were sulfoconjugated, whereas the bulk was glucoconjugated.

In summary, we have demonstrated in a controlled study that E_3 is a biologically potent estrogen. We further demonstrated that, depending on the route of E_3 administration, one will find different metabolites in the plasma. However, it appears that the biologic activity of E_3 as measured by suppression of gonadotropins is due mainly to the levels of unconjugated E_3 and not to the levels of the various conjugates. Because of the very effective conjugation of orally absorbed E_3, it appears that larger doses of E_3 would have to be given orally than vaginally to achieve an equivalent effect. Whether estrogen replacement therapy with E_3 would be safer than or as effective as other estrogen preparations remains to be established.

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Comparative evaluation of two methods of natural family planning in Colombia

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Results of a comparative study of the ovulation method (OM) and symptothermal method (STM) of natural family planning in Colombia are presented. Recruitment of volunteer couples began in August, 1976, and continued through December, 1978, during which time 566 couples were randomly assigned to one or the other of the two methods. The study included 3 to 5 months of training in the method assigned, after which the couples entered the follow-up phase of the study. They remained in follow-up until (1) they dropped out or (2) the study closed in June, 1979. Total dropout rates were high for both methods of natural family planning. One year after entry into the follow-up phase of the study, net pregnancy rates were 24.2% for OM users and 19.8% for STM users. Gross pregnancy rates were 29.2% for OM and 26.1% for STM. Differences in pregnancy rates between the two methods were not statistically significant. (AM. J. OBSTET. GYNECOL. 138:1142, 1980.)

NATURAL METHODS of family planning are defined as techniques for achieving or avoiding pregnancy by observing naturally occurring signs and symptoms of the fertile and infertile phases of the menstrual cycle.

Research in methods of natural family planning (NFP) started with the investigations of Ogino[9] and Knaus[7] who described the basic principles of the so-called rhythm or calendar method based upon length of previous menstrual cycles.

As a result of better knowledge of the physiology of the menstrual cycle, more reliable indicators of the fertile phase were later developed. It has been postulated by NFP advocates that most women can now be taught how to determine the days of the cycle when conception may result from intercourse.

The two principal methods of NFP currently in use are the ovulation method (OM) and the symptothermal method (STM). The OM as used in this study was based upon the atlas published by Billings,[2] in 1973, which takes into consideration the detection of individual daily changing patterns in the cervical mucus. The STM as used in this study is based upon Thyma's[13] double-check method, which includes record keeping of the following items: (1) basal body temperature, (2) daily changing pattern in cervical mucus, (3) symptoms such as breast tenderness, low abdominal pain, or spotting prior to or during ovulation, and (4) length of the six previous menstrual cycles.

The successful use of natural methods depends upon identification of signs and symptoms of potentially fertile days during the menstrual cycle and abstinence from intercourse or genital contact during these days.

There have been a few published studies on NFP in Colombia. Previous studies involving rhythm methods

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Supported by Special Programme of Research in Human Reproduction, World Health Organization, Contract No. H9-181-258.

Received for publication April 4, 1980.

Accepted August 18, 1980.

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revealed wide-ranging pregnancy rates. Between 1963 and 1968, Guerrero and Lores[3, 4] carried out a study with the STM (calendar plus basal body temperature) in Cali, Columbia. They observed 291 women for more than 7,072 menstrual cycles and reported a Pearl pregnancy rate of 10 pregnancies per 100 woman years of exposure. In 1968, Jaramillo-Gomez and Londoño[5] reported their experience with 701 Colombian women who used the rhythm method exclusively. They reported a Pearl rate of 47 per 100 woman years of exposure. Rice and associates[11] reported on 117 couples in Colombia totaling 2,153 months of exposure on the STM. They calculated 19.3 pregnancies per 1,300 cycles (or 100 woman years).

The dissimilar results found in Colombia and in other communities[4, 11, 12, 14] with the OM and STM, plus the dearth of studies comparing the effectiveness of these methods, led the Colombian Government and Javeriana University to request the World Health Organization to sponsor a comparative study of the OM and STM of NFP. The unique feature of this study was random assignment of volunteers into the OM and STM study groups. This procedure controls selectivity bias and seeks to balance the two groups with respect to variables which could make a difference in pregnancy rates, such as age, motivation, coital frequency, etc.

The main objective of this study was to compare the use-effectiveness of the OM and STM, as determined by pregnancy rates, at specified time intervals. Secondary objectives were to describe characteristics of volunteers who entered training and characteristics of those couples who did and did not drop out of the study and to establish reasons for dropping out.

Statistical design of the study and analysis of the data were carried out by the Department of Biostatistics, University of North Carolina at Chapel Hill.

Material and methods

After a careful selection of well-trained teachers for both methods of NFP, promotion of the study began in August, 1976, by means of the mass media, lectures, distribution of pamphlets describing the study, and home visits. Two cities, Bogotá and Palmira, were selected as sites of the study. Recruiting of couples continued through December, 1978, during which time 566 couples had met eligibility requirements and were randomly assigned to one or the other of the two methods under study. Eligibility requirements included: physical examination of couple, women between the ages of 18 and 39, couple not desiring pregnancy in the next 2 years, accidental pregnancy not be viewed as a tragedy by the couple, reluctance to engage in intercourse during fertile days of the cycle, evidence of a stable union, willingness to participate in the study for 12 to 24 months and willingness to practice the method assigned.

At randomization each couple was assigned to a particular method and enrolled in a training program to learn details of that method, as well as record-keeping activities associated with it. The training program usually lasted from 3 to 5 months. Volunteers who successfully completed training in a nonpregnant state entered the formal study (follow-up phase). They remained in the follow-up phase until (1) they dropped out or (2) the study closed in June, 1979. While in the follow-up phase, volunteers were visited monthly by study personnel to determine pregnancy status, to discuss problems in using the method, and to offer counseling as needed.

Women in the study kept a daily record of mucus description, basal body temperature (STM users only), "symptoms" such as breast tenderness, and timing of sexual intercourse. These records were submitted to study personnel monthly for review as a basis for counseling.

Results

Characteristics of total participants. The main characteristics of randomized couples at the time of admission were as follows: mean age of women was 27.2 years while the mean age of men was 31.8. The couples had, on the average, lived together for 6.5 years and had 2.2 living children. Most of the couples had attained primary or secondary school education with relatively few having attended a university. Most of the couples were married Catholics. The women were generally not employed outside the home. About half of the couples wanted more children at some time in the future but not within the next 2 years. The other half wanted no more children. Eighty percent of the women and 70% of the men felt very strongly against having sexual relations during fertile periods of the cycle. Should they become pregnant, however, 80% of the couples indicated that they could handle the pregnancy without much difficulty. The couples reported having sexual relations an average of 7 times per month.

Differences in characteristics of OM and STM groups. Mean values for nine characteristics of OM and STM couples in four distinct categories are shown in Table I. The categories are: total randomized, dropouts during training, total entering follow-up, and dropouts during follow-up.

Comparisons were made between means of OM and

Table I. Characteristics of total participants randomized, dropouts during training, total entering follow-up, and dropouts during follow-up, by method

Characteristic	*Total randomized*			*Dropouts during training*		
	1. OM	*2. STM*	*3. Total*	*4. OM*	*5. STM*	*6. Total*
No. of participants	279	287	566*	149	176	325*
Age of wife	26.9†	27.5	27.2	26.4	26.7	26.6
Age of husband	31.5	32.2	31.8	30.9	31.5	31.2
No. of prior pregnancies	2.4	2.9	2.7	2.3	2.9	2.6
No. of living children	2.0	2.4	2.2	1.9	2.3	2.1
Years living together	5.9	7.0	6.5	5.3	6.7	6.1
Sex relations (No./month)	7.1	6.8	7.0	7.3	7.2	7.2
Age at menarche	13.4	13.5	13.5	13.4	13.4	13.4
Average length of menstrual cycle (days)	29.2	29.3	29.3	29.3	29.3	29.3
Length of shortest cycle	27.1	27.0	27.1	27.5	27.0	27.2

*Includes three women who became pregnant before training began: 2 OM, 1 STM.
†Mean value.

Table II. Number and percent of dropouts during training and follow-up phase by method and reason

Reason	*Training period*			*Follow-up phase*		
	OM	*STM*	*Total*	*OM*	*STM*	*Total*
Total couples entering	279	287	566*	130	111	241
Total dropouts:	149 (100†)	176 (100%)	325* (100%)	86 (100%)	82 (100%)	168 (100%)
Pregnancy	40 (27)	39 (22)	79 (24)	32 (37)	25 (30)	57 (34)
Lack of interest or dissatisfaction with method	40 (27)	48 (27)	88 (27)	23 (27)	21 (26)	44 (26)
Relocation	26 (17)	17 (10)	43 (13)	5 (6)	10 (12)	15 (9)
Violation of rules	11 (7)	32 (18)	43 (13)	1 (1)	1 (1)	2 (1)
Vaginal discharge or mucus problems	11 (7)	3 (2)	14 (4)	—	—	—
Desire for pregnancy	6 (4)	8 (5)	14 (4)	18 (21)	19 (23)	37 (22)
Menstrual problems	3 (2)	10 (6)	13 (4)	—	1 (1)	1 (1)
Loss of partner	3 (2)	5 (3)	8 (3)	5 (6)	5 (6)	10 (6)
Other	9 (6)	14 (8)	23* (7)	2 (2)	—	2 (1)

*Includes three women who became pregnant before training began: 2 OM, 1 STM.
†Percentages are shown in parentheses.

Table III. Net cumulative proportion of couples dropping out during first year following the beginning of training period, by reason for dropout and method

Ordinal month	Women exposed at start of month	*Reason for dropout*								
		All reasons	*Pregnancy*		*Voluntary withdrawal*		*Involuntary withdrawal*		*Relocation or LTF**	
		Proportion	*Proportion*	*SE*	*Proportion*	*SE*	*Proportion*	*SE*	*Proportion*	*SE*
OM:										
1	277	0.022	—	—	0.014	0.007	—	—	0.007	0.005
3	257	0.199	0.058	0.014	0.090	0.018	—	—	0.051	0.013
6	157	0.532	0.148	0.021	0.257	0.026	0.029	0.010	0.098	0.018
9	102	0.659	0.199	0.023	0.312	0.028	0.047	0.013	0.101	0.019
12	75	0.721	0.222	0.024	0.343	0.031	0.047	0.013	0.109	0.020
STM:										
1	286	0.038	—	—	0.031	0.010	—	—	0.007	0.005
3	248	0.238	0.052	0.013	0.143	0.021	—	—	0.042	0.012
6	142	0.605	0.133	0.020	0.301	0.028	0.112	0.017	0.059	0.014
9	90	0.697	0.157	0.021	0.350	0.030	0.115	0.018	0.074	0.016
12	70	0.767	0.191	0.024	0.384	0.032	0.115	0.018	0.077	0.016

*Lost to follow-up.

Total entering follow-up			Dropouts during follow-up		
7. OM	8. STM	9. Total	10. OM	11. STM	12. Total
130	111	241	86	82	168
27.5	28.6	28.0	27.0	28.3	27.6
32.1	33.4	32.7	31.0	32.9	31.9
2.5	2.9	2.7	2.4	2.9	2.6
2.1	2.4	2.3	2.0	2.4	2.2
6.5	7.5	7.0	6.3	7.3	6.8
6.8	6.3	6.6	7.3	6.5	6.9
13.4	13.7	13.5	13.3	13.6	13.5
29.0	29.4	29.2	29.1	29.5	29.3
26.6	27.2	26.9	26.6	27.3	27.0

STM couples in each of the four categories for each of the nine variables except length of shortest cycle. With regard to the total randomized category, significant differences between OM and STM couples were found for three variables: number of prior pregnancies, number of living children, and years living together (see columns 1 and 2). With regard to the "dropouts during training" category, significant differences between characteristics of OM and STM couples were found for the same three variables: number of prior pregnancies, number of living children, and years living together (see columns 4 and 5). In other words, the STM couples who began training had lived together longer than OM couples on the average and had more children. However, during the training period, this excess of STM couples living together longer and with more children vanished. The result was that there were no significant differences in means of OM and STM couples entering the follow-up phase (columns 7 and 8). Similarly, no significant differences were found between means of OM and STM couples who dropped out during the follow-up phase (columns 10 and 11).

Reasons for dropout during training and follow-up phase.

Training period. The dropout rate was unexpectedly high—57% of trainees–during the training period of 3 to 5 months. Principal reasons for dropout were lack of interest or dissatisfaction with the method, pregnancy, relocation, and violation of rules. Details are shown in Table II.

Follow-up phase. During the course of the study, 241 couples successfully completed training and entered the follow-up phase. At the close of the study 73 couples were still practicing the method assigned to them. Reasons for dropout were recorded for the 168 couples who dropped out during the follow-up phase and are shown in Table II. Pregnancy was the leading cause of dropout, followed by lack of interest or dissatisfaction with the method and desire for pregnancy.

Cumulative dropout rates

This section is concerned with cumulative probabilities of dropout for various reasons during the first year measured from two points in time: (1) beginning of the training period and (2) beginning of the follow-up phase following successful completion of training. Rates shown in this section (except for the Pearl index) are life-table rates and the method employed is basically the approach described by Potter.[10, 15] In the preparation of life tables, allowance was made for four types of decrement: pregnancy, voluntary withdrawal, involuntary withdrawal, and relocation or lost to follow-up. Pregnancy was diagnosed by study management at Javeriana University. Voluntary withdrawal covered a variety of reasons, including lack of interest, dissatisfaction with the method, desire to become pregnant, menstrual problems, etc. Involuntary withdrawal usually meant termination by study management due to protocol violations. Relocation or lost to follow-up meant moving out of the area or otherwise becoming impossible to find.

Cumulative dropout rates measured from beginning of training. The multiple-decrement life-table approach by definition allows for the presence of competing risks. "Net" rates of pregnancy, voluntary withdrawal, involuntary withdrawal, and relocation or lost to follow-up are additive, and their sum is the dropout rate for all reasons. "Gross" pregnancy rates are useful for estimating what the pregnancy rates might have been with the assumption that there was only a single cause of decrement (pregnancy) and that all competing risks (voluntary withdrawal, etc.) were nonexistent. In the absence of competing risks, all women remain exposed to the risk of pregnancy until it occurs.

Net cumulative proportions of couples dropping out (and standard errors) for each of these four reasons and for all reasons combined, are shown in Table III by 3-month intervals.

Total dropout rates at the end of 12 months following enrollment in training were high for both OM and STM couples. Table III shows that 72.1% of OM users and 76.7% of STM users had dropped out by the end of the first year following the beginning of training.

Voluntary withdrawal was the major reason for dropout among users of both methods. Among OM users, 34.3% of couples voluntarily dropped out during the first year compared with 38.4% of STM users.

Pregnancy was the next most important reason for dropout during this 12-month period. The net preg-

Table IV. Net cumulative proportion of couples dropping out during first year of follow-up, by reason for dropout and method

Ordinal month	Women exposed at start of month	Reason for dropout								
		All reasons	Pregnancy		Voluntary withdrawal		Involuntary withdrawal		Relocation or LTF*	
		Proportion	Proportion	SE	Proportion	SE	Proportion	SE	Proportion	SE
OM:										
1	130	0.023	—	—	0.015	0.011	—	—	0.008	0.008
3	117	0.169	0.085	0.024	0.062	0.021	0.008	0.008	0.015	0.011
6	89	0.317	0.147	0.030	0.132	0.029	0.016	0.011	0.023	0.013
9	72	0.434	0.197	0.034	0.191	0.035	0.016	0.011	0.031	0.015
12	47	0.601	0.242	0.039	0.303	0.042	0.016	0.011	0.040	0.018
STM:										
1	111	0.009	—	—	0.009	0.009	—	—	—	—
3	106	0.108	0.036	0.018	0.054	0.021	—	—	0.018	0.013
6	83	0.309	0.091	0.027	0.164	0.036	0.018	0.013	0.036	0.018
9	66	0.440	0.138	0.033	0.230	0.039	0.018	0.013	0.055	0.022
12	50	0.530	0.198	0.040	0.260	0.041	0.018	0.013	0.055	0.022

*Lost to follow-up.

Table V. Pearl pregnancy rates by method

Time	OM			STM		
	Pregnancies	Woman months of exposure	Pearl rate	Pregnancies	Woman months of exposure	Pearl rate
One year from beginning of training	61	1,967	37.2	54	1,882	34.4
One year from beginning of follow-up phase	30	1,064	33.8	21	969	26.0

nancy rate was 22.2% for OM users and 19.1% for STM users. This difference is not significant.* The 12-month gross pregnancy rate was 33.6% among OM users and 33.0% among STM users. This difference is also not significant.

Relocation/lost to follow-up rates were relatively low for both OM and STM users—10.9% for OM and 7.7% for STM. Dropout rates because of involuntary withdrawal were 4.7% for OM users and 11.5% for STM users.

It should be emphasized that the dropout rates shown in Table III reflect what happened during the 12-month interval measured from the beginning of training. It includes the entire training period for all women and, for some women, several months of exposure following successful completion of training.

Cumulative dropout rates measured from beginning of follow-up phase. Net cumulative proportions of couples dropping out (and standard errors) for reasons of pregnancy, voluntary withdrawal, involuntary withdrawal, and lost to follow-up are shown in Table IV by 3-month intervals.

Total dropout rates at the end of 12 months following entry into the formal study were slightly higher for OM than STM users. Table IV shows that 60.1% of OM users and 53.0% of STM users had dropped out by the end of the first year from the beginning of the follow-up phase.

Voluntary withdrawal was again the major reason for dropout among users of both methods. Among OM users, 30.3% voluntarily dropped out during the first year compared with 26.0% of STM users.

Pregnancy was the next most important reason for dropout during this period. The 12-month net pregnancy rate was 24.2% for OM users and 19.8% for STM users. This difference is not significant. The 12-month gross pregnancy rate was 29.2% for OM users and 26.0% for STM users and, again, the difference is not significant.

Relocation/lost to follow-up rates were 4.0% for OM users and 5.5% for STM users. Dropout rates because of involuntary withdrawal were negligible for users of both methods.

Pearl pregnancy rates. Pearl pregnancy rates are very common in the literature of family planning studies, although, in general, they have limitations which

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*The test statistic used was:

$$z = P_1 - P_2 / \sqrt{(SE)_1^2 + (SE)_2^2}$$

where P_1 and P_2 are cumulative pregnancy rates of the two groups (OM and STM) and the SEs are standard errors of the respective cumulative pregnancy rates.

life-table methods do not have. For purposes of comparison Pearl rates are presented in Table V for the 12-month period following the beginning of training and for the 12-month period from the beginning of the follow-up phase for each of the two methods of NFP. The rates are based on the following formula:

$$\text{PPR} = \frac{\text{Number of pregnancies}}{\text{Woman months of exposure}} \times 1{,}200.$$

This formula expresses pregnancy rate per 100 woman years of exposure.

Measured from the beginning of training, the Pearl pregnancy rate was 37.2 pregnancies per 100 woman years of exposure for OM users and 34.4 for STM users. Measured from the beginning of the follow-up phase, the Pearl rate was 33.8 pregnancies per 100 woman years of exposure for OM users and 26.0 for STM users.

The results of this study show relatively high pregnancy rates for both OM and STM users and no significant difference between them. Total dropout rates during training and follow-up were also high.

The pregnancy rates of this study are within the ranges found in some studies of NFP[8, 14] but are higher than rates for OM recently published by Klaus and associates[6] and Ball[1] and also higher than rates for STM recently published by Rice and associates,[11] Rötzer,[12] and Wade and associates.[16]

Natural methods are behavioral methods, and the use-effectiveness rates are probably related directly to the degree of motivation of the couple to observe coital abstinence and to follow the instructions of the method.

The results of this study indicate that these two methods of NFP cannot be considered as simple methods of family planning but as a new way of life which requires changes in sexual behavior that cannot be achieved in a short period of time. Couples who participated in this study had various reasons for doing so. Their reasons varied from moral, philosophical, medical, and psychological to the simple desire of avoiding artificial means of contraception. The degree of motivation toward following periodic abstinence is related to cultural factors that make abstinence difficult to practice in Colombia.

The results of this study raise serious questions about the demand, effectiveness, and place of NFP in Colombia. Nevertheless, since there are some couples who can successfully use these methods, they should be presented with frank recognition of the limitations as well as the advantages of the methods as alternatives in the field of family planning.

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Early primary diagnosis of ovarian cancer and detection of recurrence by serum cystine aminopeptidase assay

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The aim of this study was to correlate the relationship between serum cystine aminopeptidase (CAP) activity in normal patients and that in patients with primary ovarian cancer or recurrence. Sera obtained from 63 patients and evaluated for CAP activity by the method of Babuna and Yenen[1] were grouped as follows: in 20 normal, nonpregnant patients, the mean activity was 25; in 14 patients with fibromas of the uterus, it was 38; in two patients with ovarian sarcoma, the activities were "normal" (24 and 27); and in 27 patients with primary ovarian adenocarcinoma or recurrence, the activity corresponded to the size of the tumors. Assay of serum CAP activity is a simple procedure which can be performed in any laboratory. It is a quick method for qualitative, and even quantitative, assessment of an ovarian adenocarcinoma in the nonpregnant patient. It may prove to be especially valuable in the follow-up of patients with ovarian cancer. (AM. J. OBSTET. GYNECOL. 138:1148, 1980.)

THE PROGNOSIS for ovarian carcinoma remains generally dubious in spite of all advances in treatment. The most important reason for this is the absence of methods of early detection, such as are available for carcinoma of the cervix. Diagnosis of (International Federation of Gynaecology and Obstetrics) Stage IA ovarian carcinoma is still rare; in more than half of all cases, diffuse peritoneal carcinosis is present before treatment is begun.

Numerous authors[2, 4–8, 10, 11] have tried to identify and quantify tumor-specific substances in blood, urine, or homogenized specimens of tumor tissue. In 1977, Blum and Sirota[3] observed elevated levels of cystine aminopeptidase in the urine of patients with uterine fibromas, and even higher levels in women with ovarian adenocarcinoma, in comparison to values in healthy women. On the other hand, cystine aminopeptidase activity rises in pregnant women,[1] exceeding the highest levels observed in healthy nonpregnant women by the twelfth week of gestation.

The aim of this study was to measure the activity of cystine aminopeptidase in serum, thus bypassing renal variables. The values were related to the mass of the tumor.

Material and methods

Sera were obtained and evaluated for cystine aminopeptidase activity from 63 patients grouped as follows (Table I). There were 20 normal, nonpregnant patients (28 to 70 years old). Fourteen patients had fibromas of the uterus (mean diameters, 9 cm to 15 cm). Two patients had ovarian sarcoma. Twenty-seven patients had primary epithelial ovarian cancer or a recurrence: serous cystadenocarcinoma (17); mucinous cystadenocarcinoma (three); endometrioid adenocarcinoma (one); and undifferentiated carcinoma (six).

We used the method of Tuppy and Nesvadba[13] modified by Babuna and Yenen[1] for evaluating cystine aminopeptidase serum activity. It is a relative simple colorimetric procedure based upon the hydrolysis of L-cystine-di-β-naphthylamide by the serum aminopeptidase with production of β-naphthylamine, a colorless product.

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Supported in part by the German Research Foundation (Ka 477/531/78).

Received for publication May 8, 1980.

Revised July 16, 1980.

Accepted July 22, 1980.

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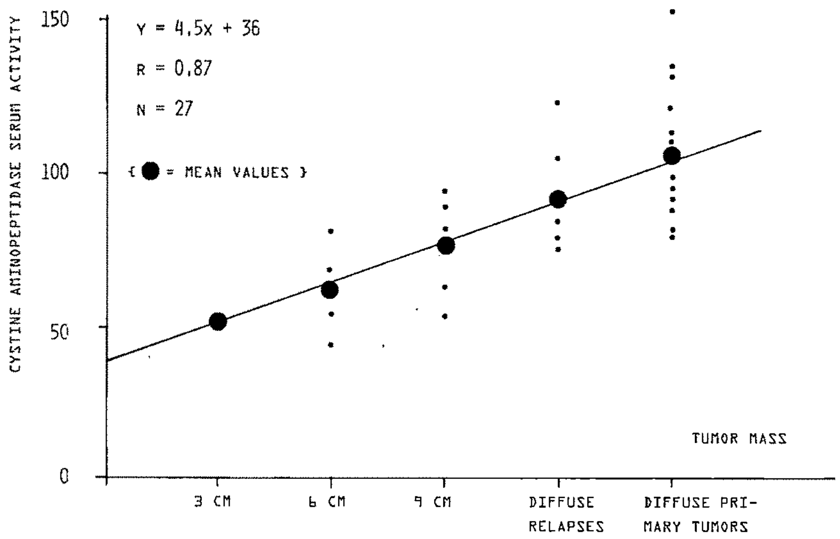

Fig. 1. Cystine aminopeptidase activity in cases of ovarian adenoneoplasm or relapse.

Table I. Cystine aminopeptidase activity in healthy, nonpregnant patients, patients with fibroma of the uterus, and patients with ovarian adenoneoplasm or relapse

	N	*Mean value*	*Range*	*Standard deviation*
Normal nonpregnant patients	20	25	10-41	11
Patients with fibromas of uterus	14	38	21-54	12
Patients with ovarian adenoneoplasm or relapse:				
Tumor mass				
3 cm	1	49	—	—
6 cm	4	62	47-78	12
Diffuse relapses	5	92	69-123	20
Diffuse primary tumors	12	108	73-155	24

Results

We obtained the following values (Table I). The healthy, nonpregnant patients had a mean serum cystine aminopeptidase value of 25. Neither patient with sarcoma had elevated activity: 24 and 27. The enzyme activity in the patients with fibromas was slightly enhanced at 38. The highest levels occurred in patients with the largest fibromas. In patients with ovarian epithelial cancer the serum cystine aminopeptidase activity corresponded to the mass of the tumors. In a case of ovarian carcinoma 3 cm in mean diameter, which was found by chance during vaginal hysterectomy, the enzyme activity was 49; in four cases of recurrent tumors 6 cm in mean diameter, it was 62; in five cases of recurrent tumors 9 cm in mean diameter, it was 71; and in five cases of diffuse recurrent tumors, it was 92.

In 12 patients with a primary, untreated epithelial cancer of the ovary with diffuse peritoneal carcinosis, we observed a mean enzyme activity of 108.

Thus, the enzyme levels were proportional to the mass of the tumor (Fig. 1). The relationship can be expressed as the function

$$y = 4.5x + 36$$

where y is the mean serum cystine aminopeptidase activity, and x is the mean tumor diameter in centimeters.

Cystine aminopeptidase activities after therapy. Eleven of our 13 patients with primary tumors underwent total hysterectomy plus bilateral adnexectomy. The other two patients and the 14 patients with recurrent tumors received cytostatic treatment. The cystine aminopeptidase activity decreased in the first group from a preoperative mean value of 102 to 58 at 5 days postoperatively, to 37 after 10 days, and to 31 after 3 months. In the second group, 12 of the 16 patients had a decrease in the enzyme activity of about 20% to 30% of the initial values at 2 days after termination of cytostatic treatment (12 gm of cyclophosphamide in 6 weeks). In the other four patients, the enzyme values increased even more; these tumors were not influenced by cytostatic treatment, so that, clinically, we observed tumor extension.

Comment

Serum cystine aminopeptidase activities in excess of 40 should be regarded with suspicion.[9] In fact, only three possibilities exist which can explain such elevations in the nonpregnant patient: (1) *a fibromatous uterus*, which must have a mean diameter greater than 9 cm to account for such enzyme levels, and is easy to diagnose clinically; (2) *an adenocarcinoma of an extragenital organ* (Epithelial tumors of the stomach, colon, gall ducts, and pancreas have been known to produce considerable amounts of peptidases on occasion,[12] but mostly leucine aminopeptidase. An increase in cystine aminopeptidase activity in extragenital tumors has not yet been observed, to our knowledge. Since this cannot be excluded a priori, these organs should be checked in the event the third possibility can be excluded.); and (3) *an adenocarcinoma of the ovary*.

Although not absolutely specific, the assay of serum cystine aminopeptidase is a very sensitive method for early detection of adenocarcinoma, especially in the ovary. We believe that this assay should be used in the primary diagnosis and in the follow up of such tumors. The development of a simpler assay which is commercially available is still being awaited.

In cases of elevated peptidase activity which cannot be explained by pregnancy or a fibromatous uterus, we recommend diagnostic laparoscopy, possibly with biopsy, even when palpatory findings are negative.

Later, an understanding of cystine aminopeptidase activity can be a factor in prompt initiation of surgical or cytostatic treatment in case of recurrence.

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Estradiol implants for conception control

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Oral contraceptives are associated with serious untoward effects and have been proscribed for women over 35 years of age. It was thought that the use of a natural estrogen (pellets of 25 mg of 17β-estradiol) implanted in descending doses (four, three, two, one) at 6-month intervals might cause less disturbance in metabolic parameters and side effects. Our experience with this regimen encompasses 18,480 cycles or 1,540 woman years. The corrected Pearl Index was 0.273, which compares favorably with other modes of conception control. Pellet implants proved to be quite acceptable to those who could not tolerate oral contraceptives. (AM. J. OBSTET. GYNECOL. 138:1151, 1980.)

ORAL CONTRACEPTIVES remain the most effective tool yet devised for the control of conception. However, there are many women who do not tolerate oral contraceptives, are not candidates for and do not want an intrauterine contraceptive device, and prefer some method other than mechanical barriers. Then, again, several authoritative bodies believe that it is prudent to limit the use of oral contraceptives to women under 35 years of age because of the rise in mortality figures for oral contraceptive users over that age.[1]

Emperaire and Greenblatt[2] reported that four pellets of 17β-estradiol (25 mg each) implanted subcutaneously at 6-month intervals provided excellent control of conception.[2] Because of earlier experiences in which ovulation was successfully suppressed by the use of oral conjugated estrogens in a monthly step-down fashion, i.e., 5 mg, 3.75 mg, 2.5 mg, 1.25 mg,[3] we decided to try a similar scheme; the number of pellets were reduced every 6 months, i.e., four, three, two, one.[4] Of course, it is mandatory that an oral progestogen be employed for 7 to 10 days each month in order to induce orderly periods of uterine withdrawal bleeding.

Material and methods

The pellets were implanted subcutaneously into the abdomen, one inch above and parallel to Poupart's ligament. A total of 490 sexually active women of reproductive age, 60% of whom were under 35 years of age, participated in this study; 410 returned for three pellets, 351 for two pellets, and 295 stayed on for the full course. Most of the women in this latter group continued at the one-pellet level every 6 months for a period ranging from 1 to 8 years. Table I shows that our experience encompassed 18,480 cycles or 1,540 woman years. The age distribution is given in Table II.

Physical examinations, including blood pressure and weight, were performed every 6 months. Blood for testing with the SMA-18 was drawn at the start and at yearly intervals. Biopsy specimens of endometrium were obtained at varying intervals to study histologic changes and also whenever abnormal bleeding occurred. Hormone assays were performed in selected patients.

Results

Endometrial studies. Because of a belief that continuous administration of estrogen is conducive to endometrial cancer, we obtained one or more biopsy specimens of endometrium in 148 women before and/or after a course of progestogen at varying intervals of

From the Medical College of Georgia and the Southeastern Research Foundation.

Received for publication July 14, 1980.

Accepted July 21, 1980.

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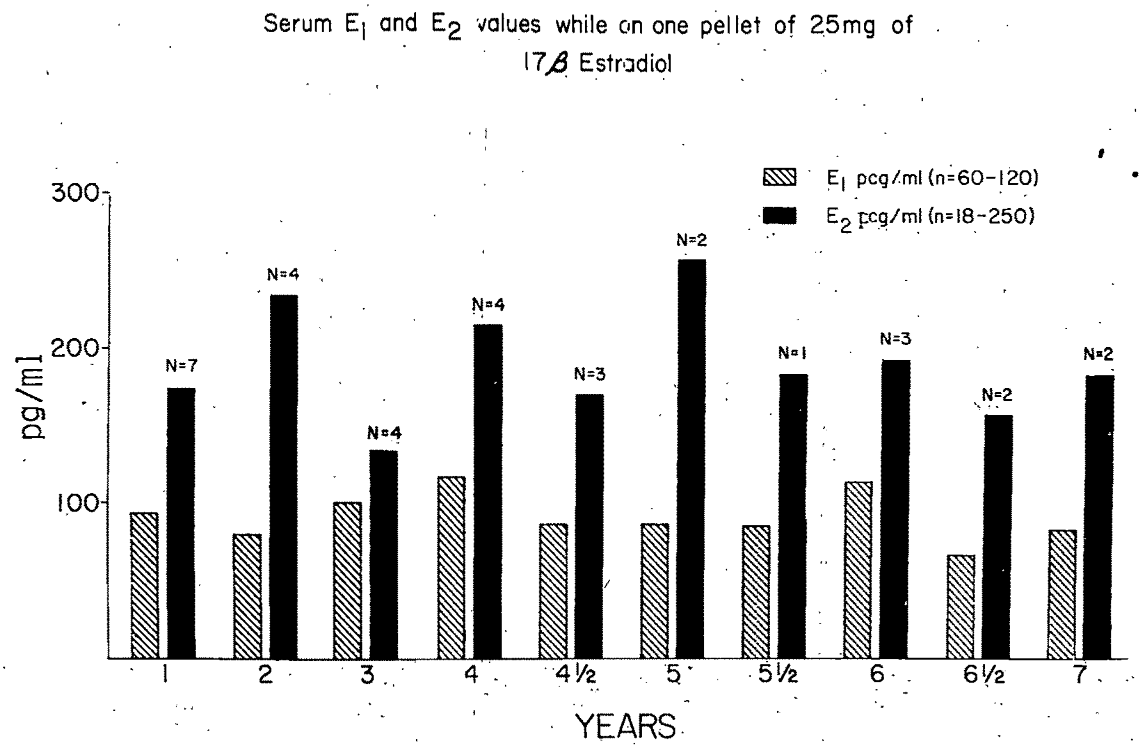

n = normal values
N = number of samples

Fig. 1. Note that serum levels of estrone and estradiol are maintained within physiologic levels on one pellet of estradiol every 6 months.

Table I. Step-down course of estradiol pellet implants in 490 females

Pellets	*No. of patients*	*Cycles*	*Woman years*
4	490	2,940	245
3	410	2,460	205
2	351	2,106	175.5
1	295	10,974	914.5
Total		18,480	1,540.0

Table II. Incidence of abnormal uterine bleeding in 490 women on hormone therapy

Age (yr)	*Total No. of patients*	*Hypermenorrhea*	*Menorrhagia*
<20	24	—	—
20-24	82	1	0
25-29	95	1	2
30-34	94	1	1
35-39	134	2	3
40-44	61	5	1
Total	490	10	7

time over a 10-year period (mean, 3.4 ± 1.9 years) (Table III). In addition, we performed 20 biopsies in 17 women who experienced hypermenorrhea (excessive withdrawal periods) or menorrhagia (breakthrough bleeding). The mean length of time after beginning the implantation of the pellets was 3.0 ± 2.4 years (Table IV). Most of the patients were on a 5-day course of progestogen (either 5 mg of norethindrone or 10 mg of medroxyprogesterone acetate). Whenever we encountered any form of hyperplasia, the duration of administration of progestogen was increased to 7 to 10 days and sometimes 13 or 14 days. The frequency of hyperplasia declined rapidly and none was found by the fourth biopsy. Two hundred sixteen endometrial biopsy specimens, obtained by suction curettage, were available for study (Table V). Of the two women who had adenomatous hyperplasia, one responded to increased doses of the progestogen with a change to a secretory endometrium; the other opted for hysterectomy.

The histopathologic characteristics of endometria of uteri from 78 women in this series of 490 was studied. Only one case of adenomatous hyperplasia was found

Table III. Intervals and frequency of endometrial biopsies for routine histologic study

Endometrial biopsy	*No. of biopsies*	*<6 mo*	*6-12 mo*	*1-2 yr*	*2-3 yr*	*3-4 yr*	*4-5 yr*	*5-6 yr*	*6-7 yr*	*7-8 yr*	*>8 yr*
First	148	9	6	28	21	24	25	16	14	3	2
Second	34	—	11	6	1	2	4	3	4	1	2
Third	11	—	2	—	3	⅞	2	—	3	—	1
Fourth	3	—	—	1	—	1	1	—	—	—	—
Totals	196	9	19	35	25	27	32	19	21	4	5

Table IV. Endometrial biopsies in women experiencing abnormal bleeding

Endometrial biopsy	*No. of biopsies*	*<6 mo*	*6-12 mo*	*1-2 yr*	*2-3 yr*	*3-4 yr*	*4-5 yr*	*5-6 yr*	*6-7 yr*	*7-8 yr*	*>8 yr*
First	17	2	3	2	4	1	1	1	1	1	1
Second	3	—	1	1	1	—	—	—	—	—	—
Totals	20	2	4	3	5	1	1	1	1	1	1

Table V. Histologic findings in 216 endometrial biopsies after pellet implantation

Endometrial biopsy	*No. of biopsies*	*Proliferative*	*Secretory*	*Mild hyperplasia*	*Moderate hyperplasia*	*Adenomatous hyperplasia*
First	165	64	44	48	7	2*
Second	37	16	11	6	4	—
Third	11	3	5	2	1	—
Fourth	3	1	2	—	—	—
Totals	216	84	62	56	12	2

*One woman with adenomatous hyperplasia opted for hysterectomy. By increasing the length of time of progestogen administration from 5 to 10 days or longer, further occurrence of hyperplasia was prevented in those who had exhibited such.

(Table VI). In all, 294 endometria were evaluated and none revealed malignant change.

Indications for hysterectomy. Hysterectomy was performed in 78 women. The mean time that elapsed from onset of therapy ranged from 3.6 years in the age group 30 to 34 years to 5 years in the age group 40 to 44 years (Table VII). The indications for hysterectomy were abnormal uterine bleeding (nine); adenomatous hyperplasia (one); fibromyomas (eight); and a palpably enlarged ovary (two). Surgical procedures in the other 58 women were performed for varying reasons: repair of rectocele or cystocele (17); adenomyosis, (nine); uterine descensus (nine); chronic hypertrophic cervicitis (four); endometriosis (six); chronic pelvic congestion and pelvic pain (three); chronic pelvic inflammatory disease (three); and a desire for permanent control of conception and continuation of estrogens without the fear of developing uterine or cervical malignancy in the future (seven).

Table VI. Histopathologic findings in the endometria of 78 patients who underwent hysterectomy

Proliferative	*Secretory*	*Mild-to-moderate hyperplasia*	*Adenomatous hyperplasia*
59	14	4	1

Influence of pellet implants on metabolic parameters. Reports have appeared which purport to show that oral contraceptives adversely influence weight, blood pressure, glucose, and lipid metabolism. Table VIII illustrates the average increase, decrease, and mean change in these parameters. The data show slight increases in all areas. Computerized statistical scattergrams for triglycerides, cholesterol, weight, glucose, and blood pressure revealed that there was no marked variation, and that overall mean change was slight. Our experience parallels that reported by Lobo and associates,[5] at the University of Southern California, on the effects of subdermal estradiol implants. They concluded that, "Unlike orally administered natural or ethinyl estrogens, parenteral estrogen replacement by E_2 pellets has minimal effects on hepatic proteins and serum lipids." Actually, their report indicates that high-density lipoproteins improved, and that overall

Table VII. Age and indications for hysterectomy in 78 women

Age (yr)	*No. of hysterectomies*	*Mean yr on therapy (range of 6 mo to 10 yr)*	*Abnormal bleeding*	*Adenomatous hyperplasia*	*Fibromyoma*	*Benign ovarian cyst*	*Miscellaneous**
30-34	21	3.6	3	—	3	—	15
35-39	35	4	2	1	5	—	27
40-44	22	5	4	—	—	2	16
Total	78	4.2	9	1	8	2	58

*Cystocele/rectocele (17); adenomyosis (nine); uterine descensus (nine); chronic hypertrophic cervicitis (four); endometriosis (six); chronic pelvic congestion and pelvic pain (three); chronic pelvic inflammatory disease (three); requested hysterectomy to eliminate possible cancer in the future (seven).

Table VIII. Increase, decrease, and mean change in metabolic parameters in 490 patients while on pellets of 17β-estradiol

Data	*Average increase*	*Average decrease*	*Mean change*
Weight (lb)	10.0	8.9	+1.1
Triglycerides (mg/dl)	62.1	59.2	+2.9
Cholesterol (mg/dl)	28.1	26.7	+1.4
Glucose (mg/dl)	18.8	17.0	+1.8
Systolic blood pressure (mm Hg)	20.1	18.3	+1.8
Diastolic blood pressure (mm Hg)	12.6	11.1	+1.5

Table X. Interval between discontinuation of pellet therapy and pregnancy

Patients	*No. pregnant*	*<12 mo*	*1-2 yr*	*2-3 yr*	*3-4 yr*
30 Desiring pregnancy*	24	8	15	1	—
1 Not desiring pregnancy	1	—	—	—	1
Totals	25	8	15	1	1

*Six conceived after Clomid and two after Clomid with human chorionic gonadotropin. Four who have not conceived have been under observation for an average of 8 months; in two others the prospects are poor because of a very low sperm count.

Table IX. Pregnancy rate in 490 fertile women receiving estradiol pellet implants

No. of patients	*Cycle*	*Woman years*	*Pregnancies*	*Pearl index*
490	18,480	1,540	4*	0.273

*Two other pregnancies occurred during the first month of therapy: one patient, it appears, was pregnant prior to implantation of the pellet, and the other conceived a few days after implantation of the pellet.

cholesterol, triglycerides, and low-density lipoproteins were unchanged.

Coagulation studies were done on 11 patients at random, and no marked changes in antithrombin III were seen. In a battery of tests, levels of factor VIII were raised, but no increase in platelet adhesiveness was found. Prothrombin time, partial thromboplastin time, platelet count, fibrinogen, and factors II, VII, X were within the normal range for our laboratory.*

Untoward effects. Seven women experienced bouts of menorrhagia. The abnormal bleeding was readily arrested by the administration of two tablets of the oral progestogen every 2 hours until bleeding was staunched (usually within 48 hours), and then two tablets per day were administered for 10 more days—a normal withdrawal period usually followed. Ten women complained of hypermenorrhea, and when the progestogen dosage was increased from 5 or 7 days to 10 or more days, excessive withdrawal periods were corrected (Table II). It is our habit now to administer progestogen for 10 days in all but a few patients.

Retention of water was not an infrequent complaint; and in some instances a diuretic and potassium were prescribed. Tenderness and fullness in the breasts were occasionally distressful to patients; an analgesic plus diuretics usually sufficed for relief. Such complaints lessened with decreasing doses. A subcutaneous hematoma at the site of implant occasionally resulted; pressure and ice bags usually alleviated the situation.

Pregnancy rate. There were six pregnancies among the 490 women of this series. Two of the pregnancies should be discounted, since one apparently occurred before the pellets were implanted, and the other occurred a few days thereafter. Patients are now advised to use caution (other contraceptive methods) during the first month. As for the other four conceptions, two

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*Courtesy of Dr. Charles Lutcher, Department of Hematology, Medical College of Georgia.

Table XI. Parity of patients who desired to become pregnant, and outcome of pregnancies after discontinuation of E_2 pellets

Age (yr)	*No. of patients desiring pregnancy*	*No. of pregnancies*	*Parity*		*Outcome of pregnancy*		
			Nulliparous	*Multiparous*	*Normal*	*Still pregnant*	*Spontaneous abortion*
20-24	9	7	4	3	6	1	—
25-29	14	12	9	3	10	1	1
30-34	6	5	—	5	4	—	1
35-39	1	1	—	1	1	—	—
Totals	30	25	13	12	21	2	2

occurred during the first 6 months, one occurred while the patient was on the second round of the one-pellet-only regimen, and the other occurred while the patient was on the fifth round. The corrected Pearl Index of four pregnancies during 1,540 woman years was 0.273, which compared favorably with other contraceptive methods (Table IX).

Future conception was not compromised. However, ovulation may not return for 6 to 12 months after discontinuation of pellet implants, and during that time the patients are advised to continue cyclic progestogen until ovulation occurs, as indicated by a Day 13 or 14 rise in basal body temperature, or is induced by the use of Clomid with or without the addition of human chorionic gonadotropin (hCG). Twenty-four of 30 women who were desirous of becoming pregnant did so within 3 years of discontinuation of therapy: eight during the first year, 15 during the second year, and one during the third year (Table X). Six required Clomid, and two required Clomid with hCG. One patient who did not wish to become pregnant conceived 46 months after discontinuing the regimen of estradiol pellets. The ages and parity of the patients and the outcome of the pregnancies are listed in Table XI.

Estradiol and estrone levels. In previous reports, we showed that estradiol and estrone were sustained at high physiologic levels on the descending-dose regimen.[4, 6] We studied the levels of estrone and estradiol in 32 women who were on the one-pellet-only regimen for 1 to 7 years. Fig. 1 indicates that the levels of estrone and estradiol remained within normal physiologic range.

Advantage of pellet implants. Women who experienced nausea, depression, sexual dysfunction, headaches, and/or acquired dysmenorrhea while on oral contraceptives usually found that such untoward reactions were rarely noticed after implantation of pellets. No instance of galactorrhea after implantation of pellets has been encountered thus far. We know of no case of pulmonary or cerebral embolus, although one patient had a questionable episode of cerebral ischemia. No endometrial cancers have resulted from this modality of conception control in an experience extending over a period of 10 years.

Comment

Pellet implants proved to be a worth-while alternative in those women who could not tolerate oral contraceptives, or who could not be relied upon to take their medication daily.

Ongoing investigations are in progress to learn more about the ratio of high-density to low-density lipoproteins and changes in coagulation factors with this form of medication. Of great interest also is the observation that, apparently, adequate cyclic oral progestogens are protective against the development of atypical hyperplasia of the endometrium and, thus, possibly prevent endometrial cancer in women who have been on continuous estrogen therapy for many years.

The pellet regimen was particularly welcome to the women over age 35, since many physicians refused to prescribe oral contraceptives for them.[6]

The favorable results noticed make us wonder whether the systemic absorption of estrogen, rather than through the gastrointestinal tract, and immediate clearance through the liver may be the explanation for decreased interference with clotting mechanisms, triglycerides, cholesterol metabolism, and glucose. Further studies are indicated to learn whether the use of a natural estrogen (E_2) induces less metabolic derangement than do the synthetic estrogens (ethinyl estradiol and mestranol).

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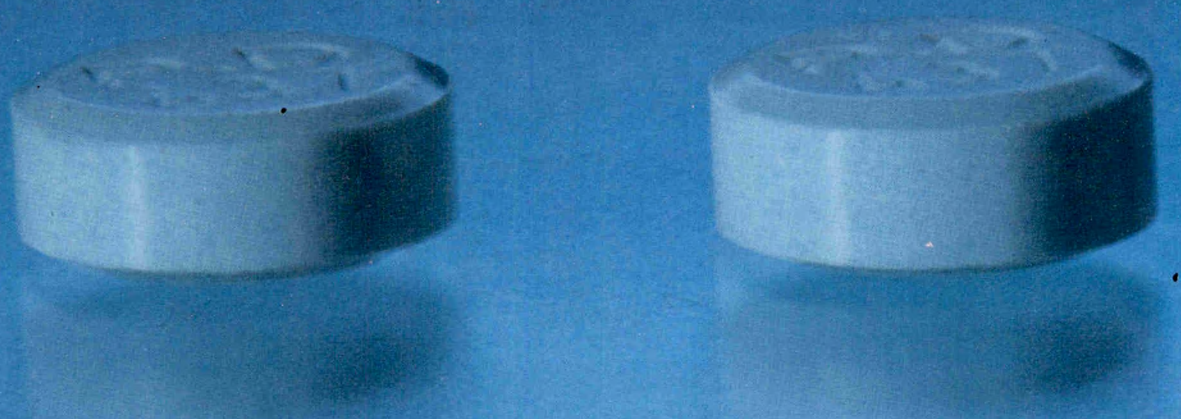

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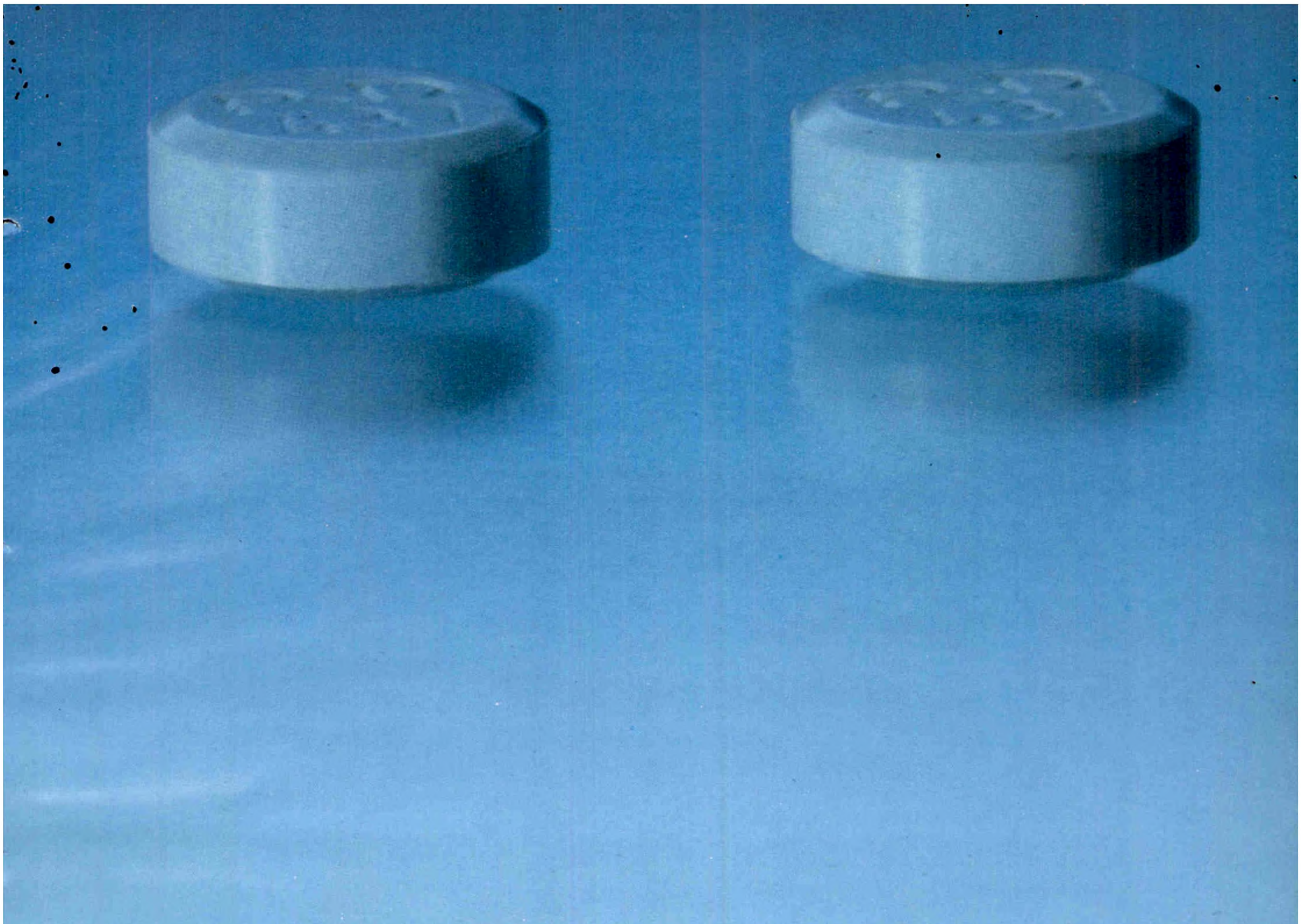

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BRIEF SUMMARY

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CAUTION

Federal law prohibits dispensing without prescription.

WARNING

1. Estrogens Have Been Reported to Increase the Risk of Endometrial Carcinoma.

Three independent case control studies have shown an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for prolonged periods. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade.

The three case control studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semiannual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration, it, therefore, appears prudent to utilize such a regimen.

Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy.

There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.

2. Estrogens Should not be Used During Pregnancy.

The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1000 exposures. Furthermore, a high percentage of such exposed women (from 30 to 90 percent) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes.

Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case control study estimated a 4.7 fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1000.

In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses.

If Estrovis (quinestrol) is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION

Estrovis (quinestrol) is available as a 100 mcg oral tablet. It is an estrogen.

Estrovis (quinestrol) is the 3-cyclopentylether of ethinyl estradiol. The chemical name is 3-cyclopentyloxy-17α-ethynyl-estra-1,3,5(10)-trien-17β-ol.

It is a white, essentially odorless powder, insoluble in water and soluble in alcohol, chloroform, and ether.

CLINICAL PHARMACOLOGY

Estrovis (quinestrol) is an orally effective estrogen as judged by conventional assay procedures employing vagina and uterine end-points in mice, rats and rabbits.

The estrogenic effects of Estrovis have been demonstrated in clinical studies by its effects on the endometrium, maturation of the vaginal epithelium, thinning of cervical mucus, suppression of pituitary gonadotropin, inhibition of ovulation, and prevention of postpartum breast discomfort.

INDICATIONS

Estrovis (quinestrol) is indicated in the treatment of: 1. Moderate to severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause, and they should not be used to treat these conditions.) 2. Atrophic vaginitis 3. Kraurosis vulvae 4. Female hypogonadism 5. Female castration 6. Primary ovarian failure

Estrovis (Quinestrol) Has Not Been Shown to be Effective for any Purpose during Pregnancy and Its Use May Cause Severe Harm to the Fetus (See Boxed Warning).

CONTRAINDICATIONS

Estrogens should not be used in women (or men) with any of the following conditions:

1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia 3. Known or suspected pregnancy (See Boxed Warning) 4. Undiagnosed abnormal genital bleeding 5. Active thrombophlebitis or thromboembolic disorders 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy)

WARNINGS

1. *Induction of malignant neoplasms.* Long-term continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. There is now evidence that estrogens increase the risk of carcinoma of the endometrium in humans. (See Boxed Warning.)

At the present time there is no satisfactory evidence that estrogens given to postmenopausal women increase the risk of cancer of the breast, although a recent long-term followup of a single physician's practice has raised this possibility. Because of the animal data, there is a need for caution in prescribing estrogens for women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease, or abnormal mammograms.

2. *Gallbladder disease.* A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladd disease in women receiving postmenopausal estrogens, similar to the 2-fold increase previously noted in users of or contraceptives. In the case of oral contraceptives the increased risk appeared after two years of use.

3. *Effects similar to those caused by estrogen-progestogen oral contraceptives.* There are several serious adverse effects oral contraceptives, most of which have not, up to now, been documented as consequences of postmenopausal estrog therapy. This may reflect the comparatively low doses of estrogen used in postmenopausal women. It would be expect that the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement are more lik to result in these adverse effects, and, in fact, it has been shown that there is an increased risk of thrombosis in m receiving estrogens for prostatic cancer and women for postpartum breast engorgement.

a. *Thromboembolic disease.* It is now well established that users of oral contraceptives have an increased risk of vario thromboembolic and thrombotic vascular diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myo infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contracepti users. There is evidence that the risk of several of these adverse reactions is related to the dose of the drug. An increase risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasibl estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk thromboembolism, or during periods of prolonged immobilization.

While an increased rate of thromboembolic and thrombotic disease in postmenopausal users of estrogens has not bee found, this does not rule out the possibility that such an increase may be present or that subgroups of women who hav underlying risk factors or who are receiving relatively large doses of estrogens may have increased risk. Therefore, estrogen should not be used in persons with active thrombophlebitis or thromboembolic disorders, and they should not be use (except in treatment of malignancy) in persons with a history of such disorders in association with estrogen use. The should be used with caution in patients with cerebral vascular or coronary artery disease and only for those in who estrogens are clearly needed.

Large doses of estrogen (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate an breast, have been shown in a large prospective clinical trial in men to increase the risk of nonfatal myocardial infarctio pulmonary embolism and thrombophlebitis. When estrogen doses of this size are used, any of the thromboembolic an thrombotic adverse effects associated with oral contraceptive use should be considered a clear risk.

b. *Hepatic adenoma.* Benign hepatic adenomas appear to be associated with the use of oral contraceptives. Althoug benign, and rare, these may rupture and may cause death through intra-abdominal hemorrhage. Such lesions have not y been reported in association with other estrogen or progestogen preparations but should be considered in estrogen use having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has also bee reported in women taking estrogen-containing oral contraceptives. The relationship of this malignancy to these drugs is n known at this time.

c. *Elevated blood pressure.* Increased blood pressure is not uncommon in women using oral contraceptives. There is now report that this may occur with use of estrogens in the menopause and blood pressure should be monitored with estroge use, especially if high doses are used.

d. *Glucose tolerance.* A worsening of glucose tolerance has been observed in a significant percentage of patients estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed while receivin estrogen.

4. *Hypercalcemia.* Administration of estrogens may lead to severe hypercalcemia in patients with breast cancer and bon metastases. If this occurs, the drug should be stopped and appropriate measures taken to reduce the serum calcium leve

PRECAUTIONS

A. General Precautions

1. A complete medical and family history should be taken prior to the initiation of any estrogen therapy. The pretreatmen and periodic physical examinations should include special reference to blood pressure, abdomen, and pelvic organs, an should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year withou another physical examination being performed.

2. Fluid retention—Because estrogens may cause some degree of fluid retention, conditions which might be influenced this factor, such as epilepsy, migraine, and cardiac or renal dysfunction, require careful observation.

3. Certain patients may develop undesirable manifestations or excessive estrogenic stimulation, such as abnormal excessive uterine bleeding, mastodynia, etc.

4. Oral contraceptives appear to be associated with an increased incidence of mental depression. Although it is not cle whether this is due to the estrogenic or progestogenic component of the contraceptive, patients with a history of depressi should be carefully observed.

5. Preexisting uterine leiomyomata may increase in size during estrogen use.

6. The pathologist should be advised of estrogen therapy when relevant specimens are submitted.

7. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice whi receiving estrogen-containing oral contraceptive therapy. If jaundice develops in any patient receiving estrogen, th medication should be discontinued while the cause is investigated.

8. Estrogens may be poorly metabolized in patients with impaired liver function and they should be administered wit caution in such patients.

9. Because estrogens influence the metabolism of calcium and phosphorus, they should be used with caution in patien with metabolic bone diseases that are associated with hypercalcemia or in patients with renal insufficiency.

10. Because of the effects of estrogens on epiphyseal closure, they should be used judiciously in young patients in who bone growth is not complete.

11. Certain endocrine and liver function tests may be affected by estrogen-containing oral contraceptives. The followin similar changes may be expected with larger doses of estrogen: a. Increased sulfobromophthalein retention. b. Increase prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet a gregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measur by PBI, T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free concentration is unaltered. d. Impaired glucose tolerance. e. Decreased pregnanediol excretion. f. Reduced response metyrapone test. g. Reduced serum folate concentration. h. Increased serum triglyceride and phospholipid concentratio

B. Information for the patient. See text of Patient Package Insert.

C. Pregnancy. See Contraindications and Boxed Warning.

D. Nursing Mothers. As a general principle, the administration of any drug to nursing mothers should be done only whe clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS

(See Warnings regarding induction of neoplasia, adverse effects on the fetus, increased incidence of gallbladder diseas and adverse effects similar to those of oral contraceptives, including thromboembolism.) The following additional adver reactions have been reported with estrogenic therapy, including oral contraceptives:

1. *Genitourinary system*: Breakthrough bleeding, spotting, change in menstrual flow, Dysmenorrhea, Premenstrual-li syndrome, Amenorrhea during and after treatment, Increase in size of uterine fibromyomata, Vaginal candidiasis, Chan in cervical eversion and in degree of cervical secretion, Cystitis-like syndrome, 2. *Breasts*: Tenderness, enlargemen secretion, 3. *Gastrointestinal*: Nausea, vomiting, Abdominal cramps, bloating, Cholestatic jaundice, 4. *Skin*: Chloasma melasma which may persist when drug is discontinued, Erythema multiforme, Erythema nodosum, Hemorrhagic eruptio Loss of scalp hair, Hirsutism, 5. *Eyes*: Steepening of corneal curvature, Intolerance to contact lenses, 6. *CNS*: Headach migraine, dizziness, Mental depression, Chorea, 7. *Miscellaneous.*: Increase or decrease in weight, Reduced carbohydra tolerance, Aggravation of porphyria, Edema, Changes in libido.

ACUTE OVERDOSAGE

Numerous reports of ingestion of large doses of estrogen-containing oral contraceptives by young children indicate th serious ill effects do not occur. Overdosage of estrogen may cause nausea, and withdrawal bleeding may occur in female

DOSAGE AND ADMINISTRATION

For treatment of moderate to severe vasomotor symptoms associated with the menopause, and for atrophic vaginiti kraurosis vulvae, female hypogonadism, female castration and primary ovarian failure:

One Estrovis (quinestrol) 100-mcg tablet once daily for seven days, followed by one 100-mcg weekly as a maintenan schedule, commencing two weeks after inception of treatment. The dosage may be increased to 200 mcg weekly if t therapeutic response is not that which may be desirable or considered optimal.

The lowest maintenance dose that will control symptoms should be chosen and medication should be discontinued promptly as possible.

Attempts to discontinue or taper medication should be made at three to six month intervals.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropria diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vagin bleeding.

HOW SUPPLIED

N 0071-0437 (P.D. 437) Estrovis (quinestrol) 100 microgram Tablets are supplied in bottles of 100 Tablets.

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OBSTETRICS

Perinatal transport to a regional perinatal center in a metropolitan area: Maternal versus neonatal transport

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A prospective study was undertaken to evaluate antenatal maternal referral, acute maternal transport, and neonatal transport to a regional perinatal center in a metropolitan area. During an 18-month period, there were 143 antenatal maternal referrals, 254 antenatal maternal transports, and 506 neonatal transports. Indications for the antenatal referrals were maternal diseases of a chronic nature. This group had a 28.7% incidence of delivery by primary cesarean section, a 15.8% incidence of low-birth weight infants, and a 30.8% incidence of neonatal admissions to the Neonatal Intensive Care Unit (NICU). The perinatal mortality rate was 13.7 per thousand which compares favorably with that of low-risk obstetric patients. Indications for the antenatal maternal transports were mainly premature labor and/or premature rupture of the membranes and third-trimester bleeding. There was a 37% incidence of delivery by primary cesarean section and a 71.5% incidence of low-birth weight infants, and 77.4% of the newborn infants were admitted to the NICU. The perinatal mortality rate was 137.4 per thousand live births. Compared to postnatal neonatal transports, the neonates transported antenatally had a slightly higher mortality rate which was not statistically significant. One hundred thirty-one neonates transported antenatally could be matched in sequential order of admission with 131 neonates transported postnatally of comparable birth weights and gestational ages. Although there was a significant increase in the incidence of delivery by cesarean section among the mothers transported antenatally, the neonates in this group had a significantly lower incidence of respiratory distress syndrome and other morbidity and a shorter hospitalization. This study suggests that antenatal referral and transport of high-risk parturient patients to a regional perinatal center may significantly decrease neonatal morbidity and length of hospitalization. (AM. J. OBSTET. GYNECOL. 138:1157, 1980.)

REGIONALIZED NEONATAL CARE has been shown to significantly decrease neonatal morbidity and mortality both in the United States and abroad.[1-6] In a survey reported by the American College of Obstetricians and Gynecologists,[7] physicians believed that regional planning of perinatal care would improve the perinatal mortality rate. It is generally believed that antenatal transport of the high-risk patient to a regional perinatal center could further decrease perinatal morbidity and mortality.[1, 8-12] Experiences reported from Arizona[1] and Tennessee[8] seem to support such a conclusion. By comparative analysis, some attempts have been made to show that indeed antenatal transport can further de-

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Received for publication June 26, 1980.

Accepted September 22, 1980.

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Table I. Indications for antenatal referral of 143 patients to Women's Hospital, January, 1978, to June, 1979

Maternal indication	*No. of patients*	%
Diabetes mellitus	33	23.1
Poor previous obstetric outcome	34	23.7
Chronic hypertension	15	10.5
Rh Sensitization	10	7.0
Pre-eclampsia	11	7.7
Incompetent cervix	9	6.3
IUGR*	5	3.5
Cardiac disease	4	2.8
Pelvic fracture/deformity	3	2.1
Seizure disorder	6	4.2
Second-trimester bleeding	3	2.1
Multiple gestation	2	1.4
Massive obesity	2	1.4
Fetal hydrocephalus	2	1.4
Drug dependency	3	2.1
Others	28	19.6

*Intrauterine growth retardation.

crease perinatal morbidity and mortality,[2, 13-15] but reported studies were experiences with a small number of patients[13-15] or were conducted in a rural setting.[2] Only the latter report showed that neonates transported antenatally had lower mortality rates than neonates transported postnatally. In a previous retrospective report, we were unable to demonstrate a difference in mortality rates although antenatal transports resulted in fewer premature neonates with morbidity.[15]

Furthermore, in most studies, no distinction has been made between high-risk maternal referrals for care and the acute high-risk maternal transport for delivery. Also, little attention has been paid to the appropriateness of the antenatal transport and whether antenatal transport resulted in the delivery of healthier neonates who did not require intensive care. Therefore, we designed a prospective study to further evaluate perinatal transport and referral to an urban regional center.

Material and methods

Memorial Hospital Medical Center of Long Beach (MHMC) with its Women's and Children's Hospitals serves as a regional perinatal center for Southern Los Angeles and Western Orange Counties. There are many communities within the catchment area and the population is approximately 2.5 million. Since 1972, MHMC has provided transport and intensive care services for more than 2,100 neonates from 50 hospitals. Twenty-six of the referring hospitals are within the catchment area of our Regional Perinatal Education Program. With the inception of a community Perinatal Outreach Program in 1976, Women's Hospital began receiving maternal referrals and acute transports of high-risk antenatal patients. The number of these antenatal referrals and transports has increased substantially from less than 60 in 1976 to 209 in 1977, 295 in 1978, and 383 in 1979. During the period from January, 1978, through June, 1979, there were 143 antenatal referrals, 254 antenatal transports to Women's Hospital, and 506 neonatal transports to Children's Hospital.

An antenatal referral was defined as a pregnant patient who was referred to Women's Hospital for continued high-risk care. Her first contact with the hospital was as an outpatient and she was eventually delivered at Women's Hospital. A maternal transport was defined as a patient who was referred to Women's Hospital on an acute basis; therefore, her first contact with the hospital was as an inpatient and the majority of these patients were delivered within a short period of time. For example, a woman with pre-eclampsia might be referred for evaluation, medical therapy and eventual delivery while a woman with severe preeclampsia might be transported for in-hospital evaluation and immediate delivery. For the purposes of the matched portion of this study, a transported neonate was defined as a newborn infant transferred to the NICU from a community hospital on the first day of life. Neonates with major congenital anomalies were excluded in the matched portion. Neonates of maternal transports were also excluded from the matched section of this study if their gestational ages were <24 weeks, if their birth weights were <500 grams, or if they had major congenital anomalies.

Medical information was recorded prospectively but all charts were re-reviewed to complete the data. From antenatal charts, the following information was collected: the mode of transport, the date of transport or referral, the date of delivery, the number of hospital admissions, the name of the transporting or referring hospital, the use of steroids to enhance fetal lung maturity, the use of tocolytic agents, antepartum testing, intrapartum fetal distress, the type of delivery, the indication for terminating the pregnancy, and the primary indication for cesarean section (if done).

Medical charts for the neonates of referred and transported mothers were also reviewed for sex, order of birth, gestational age, birth weight, Apgar scores, days hospitalized, admission to the NICU, outcome, and diagnosis.

Each of the neonates of maternal transports admitted to the NICU was paried with the next neonatal transport in sequential order, which met the criteria for a matched pair. A pair was considered matched if their birth weights did not differ by more than 200 grams and their gestational ages did not differ by more than 2

Table II. Comparison of maternal factors for referrals and transports, January, 1978, to June, 1979

	Maternal referrals		*Maternal transports*		*Total*	
	No. of patients	*%*	*No. of patients*	*%*	*No. of patients*	*%*
Cesarean section:	67	46.9	109	42.9	176	44.3
Primary	41	28.7	94	37.0	135	34.0
Repeat	26	18.2	15	5.9	41	10.3
Antepartum testing (CST*/NST†)	60	42.0	36	14.2	96	24.2
Amniocentesis	51	35.7	62	24.4	113	28.5
Betamethasone	10	7.0	77	30.3	87	21.9
Tocolytic agents:						
Alcohol	2	1.4	30	11.8	32	8.1
Magnesium sulfate	4	2.8	71	28.0	75	18.9
Ritodrine	0	–	22	8.7	22	5.5
Vasodilan	0	–	10	3.9	10	2.5
Terbutaline	0	–	3	1.2	3	0.8
Total No. of patients	143	100.0	254	100.0	397	100.0

*Contraction stress test.

†Nonstress test.

Table III. Comparison of the perinatal mortality between antenatal referrals and transports, January, 1978, to June, 1979

	Antenatal referrals	*Antenatal transports*	*Total*
No. of patients	143	254	397
Fetal death	0*	5	5
Fetal death rate (per thousand)	0	18.2	11.9
Live births	146	269	415
Birth weight <500 gm	0	12	12
Birth weight >500 gm	146	257	403
Low birth weight (<2,500 gm) (%)	23 (15.8)	202 (71.5)	225 (54.2)
No. admitted to NICU *(%)*	45 (30.8)	199 (77.4)	244 (60.5)
Neonatal death	2	31	33
Neonatal death rate (per thousand)	13.7	120.6	81.9
Perinatal death	2	36†	38
Perinatal death rate (per thousand)	13.7	137.4	93.1
Infant death	1	4	5
Death while in NICU	3	35	38

*One therapeutic abortion not included.

†Excludes live births <500 gm and infant deaths after 28 days.

weeks. Gestational age was determined by obstetric history and the neonatal assessment of Dubowitz and associates.[16] Fourteen neonates of maternal transports were not matched because of congenital anomalies and another 54 could not be successfully matched according to the above criteria. This resulted in 131 matched pairs. The chart of each matched neonatal transport was reviewed for hospital of transport, age at transport, temperature at transport, birth weight, gestational age, method of delivery, presentation at delivery, Apgar scores, maternal complications, morbidity, length of hospital stay, outcome, and diagnoses.

Results

Maternal referrals. Table I shows the indications for maternal referrals in the 143 patients referred during the study period; most of these patients were referred for medical problems which were known prior to pregnancy or for problems which were of a chronic rather than acute nature.

As indicated in Table II, 67 or 46.9% of the maternal referrals were delivered by cesarean section (28.7% incidence of primary cesarean section) and 60 (42.0%) had at least one contraction stress test. Steroids were only given to 10 (7.0%) of patients.

There were 146 live births delivered to maternal referrals, as shown in Table III, including two sets of twins and one set of triplets. Twenty-three or 15.8% of the 146 live-born infants weighed less than 2,501 grams. Twenty (13.7%) weighed more than 4,000 grams. There were no fetal deaths. One referred patient with Class R diabetes had a second-trimester induced abortion and is not included. Forty-five (30.8%) of the neonates required admission to the NICU.

Table IV. Reason for admission to the NICU for the 45 neonates of maternal referrals admitted January, 1978 to June, 1979

Reason	*No. of patients*	*Percent*
Infant of insulin-dependent diabetic mother	18	40.0
Prematurity	16	35.6
Rh sensitization	5	11.1
Congenital anomalies	2	4.4
Drug withdrawal	1	2.2
Hypoglycemia	1	2.2
Polycythemia	1	2.2
Perinatal asphyxia	1	2.2

Table V. Indication(s) for antenatal transport of 254 parturients to Women's Hospital, January, 1978, to June, 1979

Indication	*No. of patients*	*Percent*
Premature labor	141	49.1
Premature/prolonged ROM*	119	41.5
Third-trimester bleeding	39	13.6
Pre-eclampsia/eclampsia	32	12.9
Chronic hypertension	10	3.5
Diabetes mellitus	5	1.7
IUGR†	13	4.5
Multiple gestation	19	6.6
Severe polyhydramnios	6	2.1
Rh sensitization	3	1.0
Others	11	3.7

*Rupture of membranes.

†Intrauterine growth retardation.

Table VI. Diagnoses in the 35 infants of antenatal transports who died while in the NICU

Diagnosis	*No. of patients*	*Percent*
Lethal multiple congenital anomalies	10	28.6
Severe perinatal asphyxia	6	17.1
Respiratory distress syndrome	13	37.1
Severe immaturity	9	25.7
Septicemia	7	20.0
NEC* (complicated)	2	5.7
Prematurity with SIDS†	1	2.9

*Necrotizing enterocolitis.

†Sudden infant death syndrome.

Among the 45 neonates admitted to the NICU, there were three deaths, for a mortality rate of 20.5 per thousand live births.

One term infant died at 35 days of life with multiple congenital anomalies, including hydrocephalus and ambiguous genitalia. This infant's mother was referred at 33 weeks' gestation after an ultrasound examination revealed fetal hydrocephalus and polyhydramnios. One neonatal death occurred in an infant of a mother who was referred at 32 weeks and was known to be Rh sensitized. A fetal stress test showed a sinusoidal heart rate pattern. Fetal anasarca was noted on ultrasound examination. Primary cesarean section was performed immediately but the infant died on the second day of life despite intensive care. The other neonatal death occurred in a 500 gram newborn infant delivered at 26 weeks' gestation after a contraction stress test that was nonreactive positive. The mother was referred early in pregnancy because of chronic hypertension and two previous perinatal deaths. Table IV shows the reason for admission to the NICU for the 45 newborn infants of maternal referrals. It should be noted that all infants of insulin-dependent diabetic mothers are routinely admitted to the NICU in order to monitor blood glucose levels very closely.

Acute maternal transport. The indications for transport of the 254 maternal transports are shown in Table V. Premature labor and prolonged or premature rupture of the membranes are the most frequent reasons for transport. One hundred nine (42.9%) of the mothers transported antenatally were delivered by cesarean section (Table II). Sixty-two or 24.4% had an amniocentesis performed, usually to obtain a lecithin-sphingomyelin ratio and 77 (30.3%) received steroids. There were five stillbirths (Table III), two in twin pregnancies with the other twin born alive, for a fetal death rate of 18.2 per thousand. In the three other fetal deaths, a decision had been made that the fetus was too immature to survive; therefore, no active intervention was attempted. Two hundred sixty-nine neonates, including 18 sets of twins, were delivered alive to maternal transports during the study period. Twelve weighed less than 500 grams or were less than 24 weeks' gestation at birth and were judged to be too immature to survive. Fifty-eight (22.6%) of the remaining 257 neonates did not require special care and were admitted directly to the normal newborn nursery. Thirty-three or 56.9% of the these 58 neonates were delivered on the same day as the antenatal transport. Most of these were patients with premature rupture of the membranes or premature labor at 34 to 37 weeks' gestation who were delivered of infants who were large enough to be cared for in the normal nursery. The remaining 25 neonates were delivered between 2 and 91 days following transport. Seventeen of these mothers were stabilized following antenatal transport, discharged, and readmitted to deliver closer to term. Only four of the antenatal transports that resulted in well newborn infants were retrospectively judged to be inappropriate because of miscalculated gestational ages.

One hundred ninety-nine or 77.4% of the neonates required admission to the NICU. There were 31

Table VII. Birth weights and mortality rates for newborn infants of antenatal transports and neonatal transports admitted to the NICU, January, 1978, to June, 1979

	Antenatal transports				*Neonatal transports*			
	Admissions		*Deaths*		*Admissions*		*Deaths*	
Birth weights (gm)	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
501-750	16	8.0	12	75.0	12	2.4	10	83.3
751-1,000	25	12.6	8	32.0	19	3.8	7	36.8
1,001-1,250	17	8.5	1	5.9	29	5.7	12	41.4
1,251-1,500	38	19.1	6	15.8	36	7.1	4	11.1
1,501-1,750	29	14.6	2	6.9	32	6.3	3	9.4
1,751-2,000	27	13.6	3	11.1	29	5.7	1	3.5
2,001-2,250	24	12.1	1	4.1	37	7.3	1	2.7
2,251-2,500	5	2.5	0	–	61	12.1	4	6.6
Total LBW*	181	91.0	33	18.2	255	50.3	42	16.5
2,501-4,000	17	8.5	2	11.8	224	44.3	21	9.4
>4,000	1	0.5	0	–	27	5.3	0	–
Total	199	100.0	35†	17.6	506	100.0	63	12.5

*Low birth weight.

†Includes four infant deaths.

Table VIII. Comparison between matched pairs admitted to the NICU for mean hospital stay, mortality rates, primary cesarean section rates, and 5-minute Apgar scores

	Antenatal transports	*Transported neonates*	*Statistical significance**
No. matched pairs	131	131	–
Mean birth weight (± 1 SD)	1,613 ± 600	1,626 ± 605	–
Mean gestation age (± 1 SD)	31.8 ± 3.5	31.7 ± 3.4	–
Mean days in hospital for surviving pairs (± 1 SD)	28.7 ± 24.5	32.9 ± 26.3	P < 0.005
Mean days in hospital for pairs who died (±1 SD)	7.4 ± 6.7	4.7 ± 3.9	NS
No. of deaths	16	18	–
Mortality (%)	12.2	13.7	–
Primary cesarean section (%)	(38.9)	(22.7)	P < 0.025
5-minutes Apgar score			
<3	7 (5.3%)	8 (6.1%)	
4-6	20 (15.3%)	22 (16.8%)	
7-10	99 (75.6%)	76 (58.0%)	
No information	5 (3.8%)	25 (19.1%)	

*Statistical significance determined by Student's t test for paired data or chi-square test. NS: Not significant.

neonatal deaths and four infant deaths in the NICU for a mortality rate of 175.8% per thousand admissions to the NICU. Ten of the neonatal deaths were due to congenital anomalies incompatible with life, for a lethal anomaly rate of 3.9% of the 257 neonates. These ten plus four other surviving neonates with congenital anomalies (1 with Down's syndrome, 1 with gastroschisis, 1 with amniotic band syndrome, and 1 with congenital heart disease) were excluded from the matched portion of the study. The diagnoses for the 35 neonates who died while in the NICU are shown in Table VI.

In Table VII, the mortality rates by birth weight are shown for all neonates of antenatal transports admitted to the NICU and all neonatal transports during the study period. Forty-one (20.6%) of the neonates of antenatal transports compared with 31 (6.2%) of the neonatal transports weighed 1,000 grams or less at birth. This difference is significant ($p < 0.001$). The overall mortality rates of 17.6% and the 12.5% for the antenatal and neonatal transports are not statistically significantly different. Neonatal mortality is not different even when neonates with congenital anomalies are excluded.

One hundred thirty-one neonates of maternal transports were matched by birth weight and gestational age with 131 neonatal transports. In Table VIII the mean hospital stay for surviving pairs, mortality rates, primary cesarean section rates, and 5-minute Apgar scores are compared for the matched pairs. The mean hospital stay for surviving pairs and cesarean section rates differ significantly between the two groups with neonates of maternal transports having shorter hospi-

Table IX. Admitting diagnoses for the matched pairs of newborn infants transferred antenatally and neonatally and admitted to the NICU, Januray, 1978, to June, 1979

Diagnoses	*Antenatal transport*	*Neonatal transport*	*Statistical significance**
Prematurity without morbidity	35 (26.7%)	4 (3.1%)	$p < 0.005$
Respiratory distress syndrome	44 (33.6%)	82 (62.6%)	$p < 0.005$
Retained lung fluid	25 (19.1%)	23 (17.6%)	NS
Perinatal asphyxia	10 (7.6%)	19 (14.5%)	NS
Suspected septicemia	9 (6.9%)	9 (6.9%)	NS
Birth injury	2† (1.5%)	0	NS
Hemolytic disease	6 (4.6%)	1 (0.8%)	NS
Severe immaturity	3 (2.3%)	0	NS
Drug withdrawal	1 (0.8%)	1 (0.8%)	NS
Other	2 (1.5%)	2 (1.5%)	NS

*Determined by chi-square test. NS: Not significant.
†Clavicle fractures.

Table X. Birth weight, gestational age, mortality and diagnoses in the 54 unmatched neonates of antenatal transports

Diagnosis	*No. of patients*	*Percent*
Prematurity without morbidity	17	31.5
Respiratory distress syndrome	19	35.2
Severe immaturity	6	11.1
Suspected septicemia	8	14.8
Perinatal asphyxia	2	3.7
Retained lung fluid	1	1.9
Others	4	7.4

Mean birth weight (±1 SD): 1,668 ± 894 gm. Mean gestational age (±1 SD): 31.5 ± 4.5 weeks. No. of deaths: 6 (11.1%).

tal stays and more deliveries by primary cesarean sections.

In Table IX the diagnoses are indicated for the matched pairs. There is a significantly larger number of neonates with prematurity but no other morbidity ($p < 0.005$), as well as a lower incidence of respiratory distress syndrome ($p < 0.005$), among the neonates of antenatal transports.

Fifty-four neonates could not be matched by our criteria. Table X shows the mean birth weight, gestational age, diagnoses, and mortality for this group.

Comment

Regional planning of perinatal care, based on geographic and demographic characteristics of a region and the availability of trained personnel and facilities, is generally believed to improve the quality of perinatal care and to decrease morbidity and mortality. In association with rapid improvement in perinatal care during the past decade, regionalized perinatal care experiences reported from Arizona,[1] Tennessee,[8] and Massachusetts[9] have indicated that indeed such a system may lessen perinatal mortality and morbidity. In 1977, the Committee on Perinatal Health with participants from the American Academy of Family Physicians, The American Academy of Pediatrics, the American College of Obstetricians and Gynecologists, and the American Medical Association, assisted by the National Foundation–March of Dimes, published a booklet[17] outlining a regionalized system of planning for perinatal care. Experiences reported to date regarding the advantages of such regionalized systems are scant because of difficulty in designing appropriate prospective studies and because of a lack of clear data regarding improvement in perinatal care with regard to decreasing morbidity, mortality, and cost.

In a metropolitan area, ideally all parturients should be delivered in a Regional Center which can provide appropriate personnel and facilities for both expected and unexpected perinatal problems. Boehm and Haire[8] suggested that many neonatal transports to their center could have been more safely transported in utero had there been a mechanism by which the mother could be easily referred to the tertiary center prior to delivery.

When antenatal and postnatal transports are evaluated, the following items should be considered: (1) proper match of antenatal and postnatal transports.[15] (2) prospective and retrospective appropriateness of antenatal transports, and (3) differences between maternal referrals for stabilization and continuous-care and acute maternal transports. In this study, antenatal and neonatal transports were matched for birth weight and gestational age but it was not possible to match for other perinatal risk factors. With the present study, we show that only four of 254 antenatal transports were judged to be inappropriate by retrospective evaluation. One may question the appropriateness of transporting mothers who subsequently have nonviable neonates (birth weight <500 gm and gestational age <24 weeks). Prospectively it is difficult to make an appropriate decision. This study also distinguished between acute maternal transports and referrals for continued antenatal care.

Despite the above-noted limitations, neonates of antenatal transports showed a lower incidence of respiratory distress syndrome than neonatal transports with comparable birth weights and gestational ages. The lower incidence of respiratory distress syndrome may be in part due to the use of tocolytic agents to stop labor, the use of steroids to enhance lung maturity, and less asphyxia. As Merenstein and associates[14] noted, we also found that the length of hospitalization was significantly shorter for neonates of the antenatal transport. Significant numbers of antenatally transported preterm neonates were without morbidity, which may shorten the length of hospital stay and decrease the chance of long-term morbidity. This finding may be influenced by the fact that some preterm neonates without morbidity were cared for at the hospital of birth rather than being transported. This would provide a bias toward antenatal transports. On the contrary, very premature depressed neonates may not be transferred because they may be judged nonsalvageable whereas very premature vigorous neonates may be transported, providing a bias toward neonatal transports in this category because antenatal transports are not so preselected. In a recent report by Williams and Hawes[18] evaluating the factors involved in improving mortality in California, it was found that higher cesarean section rates correlated most significantly with better than expected perinatal mortality rates. Whether cesarean section delivery may be responsible for decreased morbidity in preterm infants, as suggested by Stewart and associates,[19] is a matter for debate and further studies are needed for clarification. Other factors responsible for decreased morbidity among neonates transported antenatally may be better obstetric care and a decreased number of infants with asphyxia because of electronic fetal monitoring.

Harris and associates[2] reported that neonatal mortality was lower for maternal-fetal transports than for newborn transports in groups with a similar mortality risk. This has not been our experience. As seen in Tables VII and VIII, when neonates transported antenatally are compared with the neonatal transports, no significant differences in mortality are shown between the two groups. This may be explained by the fact that mortality is a very crude index and easily influenced by neonatal intensive care. It may also be that the care provided in community hospitals, influenced by our Perinatal Outreach Program, is similar to our own care in stabilization of high-risk neonates and, finally, the distance of transport and the climate are not sufficient factors in our area to influence the outcome. A limitation to this finding is that sicker and very premature neonates may have died at referring hospitals before transport could be arranged. This bias does not occur in antenatal transports.

Other advantages of antenatal transport are that some premature labors are successfully stopped and the pregnancies are carried to term or near term, resulting in decreased neonatal morbidity and mortality. Additional benefits are that both mother and infant are in the same institution, which gives the mother the chance of interaction with her infant and the personnel caring for her infant, and she can become more familiar with the infant's problems and the clinical course. With increased support available at the regional perinatal center, most often the mother becomes familiar with the neonatal personnel prior to delivery. Obvious disadvantages of the antenatal transport are that obstetric care is provided by a physician other than the patient's private physician with whom she developed rapport and confidence, there is a lack of familiarity with the new hospital, and finally the patient may be farther from supportive family members although this is not a significant problem in a metropolitan area.

In conclusion, the apparent benefits of antenatal transport to a regional perinatal center are the decreased incidence of respiratory distress syndrome, shorter neonatal hospitalization, and decreased neonatal morbidity, but many different factors may influence these parameters and one cannot conclude that the better outcome is due only to antenatal transport.

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Surgery during pregnancy and fetal outcome

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As many as 2% of all pregnant women undergo surgery during gestation, but there are few reports of the effects of anesthesia and surgery on fetal outcome. The present paper presents information on 287 women who had surgery during pregnancy. Surgery during early pregnancy was associated with a significant increase in the rate of spontaneous abortion compared to the rate in a control group that did not have surgery. There were no differences in the incidence of congenital abnormalities in the offspring of women who had surgery during early pregnancy. The data suggest that elective surgery be deferred during early pregnancy to minimize potential fetal loss. (AM. J. OBSTET. GYNECOL. 138:1165, 1980.)

RESULTS obtained from a national study of health of dental personnel have recently been presented.[1] The present report specifically examines fetal outcome during an 11-year period in wives of dentists and in female dental assistants who underwent surgery and anesthesia during the first and/or second trimesters of pregnancy.

Methods

A questionnaire was mailed to 29,514 male dentists and 30,272 female dental assistants. A total of 21,634 (73.3%) of the dentists and 21,202 (70.0%) of the assistants completed the questionnaire which asked questions about anesthetic practice and exposure, health history and pregnancy history of the respondent, or, in the case of the dentists, the spouse for the years 1968 to 1978. The questionnaire additionally requested information concerning exposure to anesthetics for surgical procedures occurring during each trimester of each pregnancy.

Spontaneous abortion was defined as loss of the product of conception prior to the thirteenth week of gestation (first trimester) or during the fourteenth to twenty-sixth week of gestation (second trimester). Pregnancies terminated by therapeutic abortion were excluded. The rate of spontaneous abortion was defined as the number of cases per 100 reported pregnancies. The rate of congenital abnormality was based on the number of live-born babies with one or more abnormalities per 100 births. Skin abnormalities were excluded.

Occupational exposure to inhalation anesthetics was defined as paternal (maternal) exposure reported in the work history section of the questionnaire for the year prior to the computed date of conception. The nonexposure group was restricted to those reporting no inhalation anesthetic exposure in any of the study years prior to conception. All rates were standardized by the direct method and adjustments for significant covariates (maternal age, smoking history) were made. Standard errors for adjusted rates were computed with each cell regarded as a binomially distributed proportion and then averaging cells separately within those individual groups to estimate the adjusted rates and their standard errors. P values were computed by the Mantel-Haenszel method and a value of ≤0.05 was considered statistically significant.

Results

A total of 12,929 pregnancies with completed data were reported for the period 1968 to 1978. Anesthetics for surgery were administered to 187 women during the first trimester of gestation and to 100 women dur-

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Supported by Grant OH-00775 from the National Institutes of Health.

Received for publication June 16, 1980.

Accepted September 15, 1980.

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0002-9378/80/251165+03$00.30/0 © 1980 The C. V. Mosby Co.

Table I. Spontaneous abortion in dental wives and dental assistants

Group	First trimester		Second trimester	
	Rate*	P value	Rate*	P value
Control—no occupational exposure or surgery	5.1 ± 0.2 (8654)		1.4 ± 0.1 (8210)	
Occupational exposure only; no surgery	8.5 ± 0.5 (4088)	<0.01	2.6 ± 0.3 (3755)	<0.01
Surgery only; no occupational exposure	8.0 ± 3.4 (122)	0.01	6.9 ± 3.9 (54)	<0.01
Surgery plus occupational exposure	14.3 ± 3.2 (65)	<0.01	0 (46)	—

*Rates adjusted for maternal age and smoking history.

Table II. Congenital abnormalities in offspring of dental wives and dental assistants

Group	Rate*	First trimester	Second trimester
Control—no occupational exposure or surgery	4.5 ± 0.2 (8006)	—	—
Occupational exposure only; no surgery	5.0 ± 0.4 (3624)	—	—
Surgery only; no occupational exposure	—	3.1 ± 1.6 (103)	5.8 ± 4.2 (48)
Surgery plus occupational exposure	—	7.2 ± 3.3 (51)	2.3 ± 1.7 (46)

There were no significant differences for any group compared to the control.

*Rates adjusted for maternal age and smoking history.

ing the second trimester. Occupational exposure to inhalation anesthetics, either direct (dental assistants) or indirect (wives of exposed male dentists), was associated with a significant increase in the spontaneous abortion rate over that in the control group during both trimesters. Anesthesia for surgery during either the first or second trimester was also associated with increased fetal loss. There was no significant increase noted in the number of congenital abnormalities in children born to women who had surgery during pregnancy. Results are shown in Tables I and II.

Comment

It has been estimated that as many as 0.5% to 2.0% of all pregnant women undergo surgery during gestation for reasons unrelated to pregnancy.[2, 3] In our survey, 2.2% of the respondents had surgery during the first or second trimester of pregnancy. The potential hazards of anesthesia as related to interference with maternal and fetal physiology have been discussed by Pedersen and Finster.[4] In the clinical situation it is especially difficult to separate the effects of anesthesia from those of surgery. Several animal studies have demonstrated that acute exposure to anesthetics alone in varying concentrations at critical times during gestation can lead to spontaneous abortion or congenital abnormalities.[5-8] However, species variation, concentrations and types of anesthetic used, and duration of exposure differ from study to study, making interpretation of the results of these animal studies to human pregnancy difficult.

Despite the very large numbers of pregnant women requiring anesthesia, there have been few reports of fetal outcome. Two previous studies have considered the problem.[2, 3] In the first of these studies, 67 of 18,493 (0.36%) pregnant women underwent surgery.[3] Only 10 of these women had surgery during the first trimester of pregnancy, and in this group two fetal deaths occurred. Since one of the fetal deaths was associated with maternal death, the actual incidence of perinatal mortality associated with first-trimester surgery in this report was 1/9 (11.1%). The sole fetal death during first-trimester surgery was in a woman undergoing spinal anesthesia for appendectomy. Forty-five patients had surgery during the second trimester and fetal death occurred in five (11.1%). Four of these deaths followed Shirodkar procedures for cervical incompetence with threatened abortion. No fetal deaths were associated with any of the general anesthetics administered during the second trimester. No congenital abnormalities were noted in the offspring of women who had surgery during pregnancy.

The second study examined records of 9,073 obstetric patients, of which 147 (1.6%) had surgery during pregnancy.[2] These patients were compared to a control group of women who did not have surgery during pregnancy. In 47 cases, surgery was performed during the first trimester, and four fetal deaths (8.5%) occurred. The overall perinatal mortality rate in the control group, which included fetal deaths during all three trimesters, was 2.0%. In those women who underwent first-trimester surgery and subsequently were delivered of live-born offspring, the incidence of congenital birth defects was 9.3%. This was not significantly different from the 6.0% incidence of congenital abnormalities in children born to women in the control group. In the second-trimester surgery group, fetal deaths occurred in 6/58 women (10.3%). Congenital defects were noted in 6/52 (11.5%) offspring of these women. Of the 18 women who had Shirodkar procedures, the perinatal mortality rate was 33.3%. This high fetal mortality rate was not unexpected considering the indication for this surgery. Of the eight patients who underwent major extraperitoneal surgery during the first trimester with the use of general anesthesia, two (25.0%) had infants with a birth defect. However, 2/11 (18.0%) patients undergoing minor surgery (cervical biopsy without anesthesia) during the first trimester of pregnancy also were delivered of term infants with birth defects. The authors concluded that no specific anesthetic agent or technique was superior with respect to fetal outcome, and that it is the indication for surgery and not the anesthetic that is the important factor in predicting pregnancy outcome.

Our data base includes information on 187 women who had surgery during the first trimester and 100 women who had surgery during the second trimester of pregnancy. In the previous studies the control groups were not separated into trimesters in which the fetal loss occurred, whereas our control groups were divided into first- and second-trimester abortions. In addition, while earlier studies were based only on hospital records of stillbirths and did not include women who aborted outside the hospital, our data include all perinatal losses. Finally, the data we report were adjusted for maternal age and for cigarette smoking, both representing factors which influence fetal outcome.[9]

We noted a significant increase in spontaneous abortions in those women who had anesthesia and surgery during the first trimester of pregnancy. Previous occupational exposure to inhalation anesthetics (minimum of 1 year preceding pregnancy) either direct, by women dental assistants, or indirect, by the wives of dentists, was also associated with increased risk of spontaneous abortion. If the mother was directly or indirectly occupationally exposed to anesthetics and also underwent surgery during the first trimester, the risk of spontaneous abortion increased almost threefold above the control.

The absolute rate for spontaneous abortion decreased during the second trimester of pregnancy, although surgical exposure during the second trimester was associated with a proportionally greater risk for fetal loss as compared to the first trimester. Since Shirodkar procedures are performed during the second trimester, several women presumably had this operation. Thus, the association between surgery and anesthesia during the second trimester in reference to fetal loss is less clear-cut. Again, as in the first trimester, occupational exposure to anesthetic gases was associated with an increase in fetal loss above the control. No spontaneous abortions occurred in the 46 women who reported occupational exposure and surgery during the second trimester. No apparent explanation is available.

As with the previous reports,[2, 3] we found no association between surgery during early pregnancy and congenital abnormalities in live-born offspring.

The mail survey approach utilized in this study has several important limitations, which include possible responder bias, inaccurate recall of events, and incomplete return rates. In addition, information as to the type of anesthetic used and the operation performed during pregnancy was not available. Despite these acknowledged shortcomings, the present study is important in that it reports fetal outcome for a large series of women who had surgery during early pregnancy. We found a significant increase in fetal losses for women who had surgery during the first or second trimester of pregnancy. Although we and others have found no increase in the congenital abnormality rate in the offspring of women who had surgery during early pregnancy, the increased fetal loss in these women prompts support to the recommendation to defer all but urgent surgery during gestation. As in previous studies, we were unable to determine whether the anesthetic, the surgical procedure, or the combination provided the cause for the increased rates of spontaneous abortion noted.

We wish to thank J. Macon Paine for her assistance with this study.

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Evidence that fetal membranes are not involved in cortisol metabolism: Study of dichorionic twin pregnancies

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11β-Hydroxysteroid dehydrogenase (11β-HSD, E.C.1.1.1.146) activity has been measured in samples of decidua, choriodecidua, and chorion obtained from five sets of dichorionic twins, with radiolabeled cortisol and cortisone used as substrates. 11β-HSD activity was significantly higher in the decidua than in the choriodecidua, in both the oxidative (cortisol to cortisone) and reductive (cortisone to cortisol) directions (activity expressed as percentage conversion): 53.4% ± 6.7% versus 38.5% ± 7.4% ($p < 0.05$) and 21.3% ± 2.2% versus 15.3% ± 3.2% ($p < 0.025$) (mean ± SEM, $n = 10$). No measurable activity in either direction was found in the chorion obtained from the wall separating the two amniotic sacs (decidua-free chorion). In both decidua and choriodecidua oxidative activity was higher than reductive activity ($p < 0.005$ for both). These results demonstrate that 11β-HSD activity attributed to the chorion is due to decidual contamination.

(AM. J. OBSTET. GYNECOL. 138:1168, 1980.)

THE FETAL MEMBRANES have been implicated in the onset of labor in man, on the basis that these tissues are involved in prostaglandin and steroid metabolism.[1, 2] Regarding the latter, a variety of steroid-metabolizing enzymes have been described in both amnion and chorion obtained at term. These include 17β-hydroxysteroid dehydrogenase (17β-HSD),[3] 3β-HSD,[2, 4] arylsulfatase (chorion),[5, 6] aromatase,[3] 5α-reductase,[2] 20α-HSD,[2] 11β-HSD (chorion),[7] and several other steroid dehydrogenases.[8] The amnion and chorion fuse at the end of the first trimester of pregnancy[9] and thereafter remain in intimate contact with the uterine decidua until delivery. After delivery, amnion and chorion can be easily separated, but the chorion usually has a layer of decidua adherent to it,[10] which often cannot be totally removed, even by careful scraping. This makes it difficult to determine which enzyme activities are present in the chorion and which are due to decidual contamination.

We are interested in the role of human fetal membranes in corticosteroid metabolism,[11] which is regulated by 11β-HSD (E.C. 1.1.1.146). This enzyme catalyzes the interconversion of 11β-hydroxycorticosteroids (cortisol) into biologically less active 11-oxo-metabolites (cortisone), an important conversion in view of the possible involvement of cortisol in the onset of labor and fetal maturation.[12] Although 11β-HSD has been considered a chorionic enzyme,[7, 13] we present here evidence which does not support this belief. We have studied 11β-HSD in membranes obtained from dichorionic twins, where it is possible to obtain chorion totally free of decidual contamination from the wall separating the two amniotic sacs; our results show that

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This study was supported by a grant from the Fundación "Esteban Romero," University of Murcia, Spain, awarded to A. L. B.

Part of this work was presented at the One Hundred Fifty-eighth Meeting of the Society for Endocrinology, London, England, November 22-23, 1979.

Received for publication April 23, 1980.

Revised September 11, 1980.

Accepted September 16, 1980.

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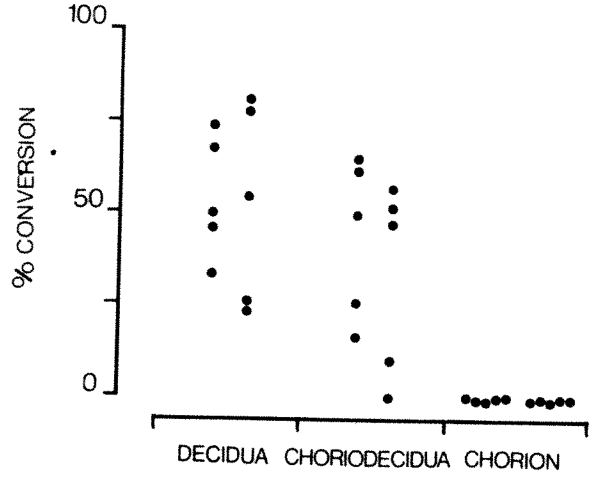

Fig. 1. Cortisol-to-cortisone conversion by homogenates of decidua, choriodecidua, and chorion obtained from dichorionic twins. Each point represents the mean of duplicate measurements. For each tissue the values on the left column correspond to the first twin and those on the right column, to the second twin. See text for further details.

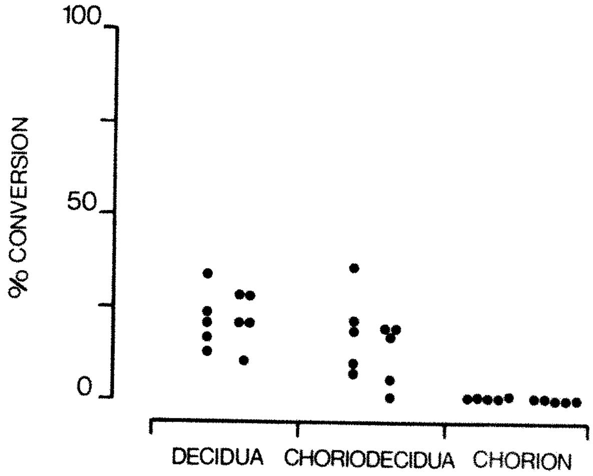

Fig. 2. Cortisone-to-cortisol conversion by homogenates of decidua, choriodecidua, and chorion obtained from dichorionic twins. Each point represents the mean of duplicate measurements. For each tissue the values on the left column correspond to the first twin and those on the right column, to the second twin. See text for further details.

11β-HSD activity is of decidual, rather than chorionic, origin.

Material and methods

[1,2-^{3}H]-Cortisol (specific activity 44 Ci/mmole, [1,2-^{3}H]-cortisone (specific activity 35.4 Ci/mmole), [4-^{14} C]-cortisol (specific activity 55 mCi/mmole), and [4-^{14}C]-cortisone (specific activity 58 mCi/mmole) were purchased from the Radiochemical Centre, Amersham, England. Purity was checked by thin-layer chromatography (TLC) and improved when necessary. Nonradioactive steroids and the cofactors nicotinamide adenine dinucleotide phosphate (NADP$^+$) and nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) were obtained from Sigma Chemical Co., St. Louis, Missouri. All solvents used were reagent grade from Fisons, Loughborough, England.

Fetal membranes were obtained from five sets of dichorionic twins. Gestational age range was from 35 to 39 weeks. Labor was of spontaneous onset in three cases and induced in two: one with oxytocin and one with prostaglandin E_2 (vaginal pessary). In all cases both infants were delivered vaginally with the use of epidural anesthesia and all were in good condition at birth. Nine were female and one was male. Placentas and fetal membranes were carefully investigated immediately after delivery. The wall separating the two amniotic sacs was identified. Only those cases where this wall consisted of two amnions and two chorions (dichorionic twins) were considered for study. Samples of decidua-contaminated chorion (choriodecidua), decidua-free chorion (chorion, obtained from the separating wall), and decidua dissected off the chorion (decidua) were taken from each sac. No samples of amnion were taken since this tissue has no 11β-HSD activity.[11, 13] The tissues were washed in saline, blotted dry, weighed, and minced before homogenization. Homogenates were prepared with a Bühler mechanical homogenizer (Bühler, West Germany) in phosphate buffer, 0.1M, pH 7.4 (1/10, w/v).

11β-HSD assay. 11β-HSD activity was measured as described elsewhere.[14] All incubations were performed in 0.1M phosphate buffer, pH 7.4. Incubations were carried out in tubes containing 1 μCi (approximately 0.025 nmoles) of [1,2-^{3}H]-cortisol or [1,2-^{3}H]-cortisone plus 2 μmoles of NADP$^+$ or NADPH and 5 nmoles of substrate in 0.5 ml of buffer. The tubes were placed in a water bath at 37° C with continuous shaking. Reactions were started by the addition of 0.5 ml of homogenate. Controls consisted of incubation medium without tissue. In these conditions reactions were linear for 15 minutes in the direction cortisol to cortisone and for 30 minutes in the reverse direction.[14] At these times, 0.4 ml duplicate samples of the incubates were taken into tubes containing 1 ml of acetone. This immediately stopped the reaction. After the acetone was evaporated in a stream of nitrogen, approximately 5,000 cpm of [4-^{14}C]-cortisol and [4-^{14}C]-cortisone was added to each tube. The tubes were extracted twice with 2.5 ml of ethyl acetate and the extracts were dried under nitro-

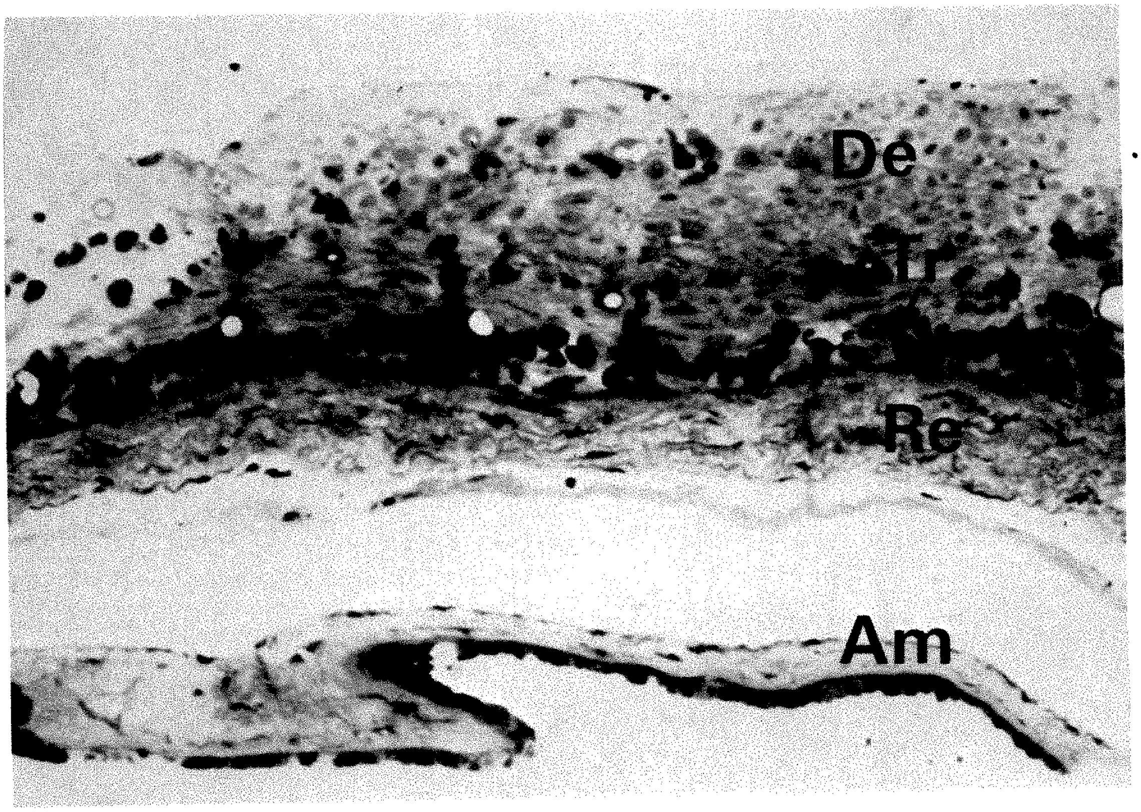

Fig. 3. Fetal membranes obtained from an area in contact with the uterine wall. Note the lack of a regular line of division between the trophoblastic cells of the chorion and the contaminating decidua. *De* = Decidua; *Tr* = trophoblastic layer of the chorion; *Re* = reticular layer of the chorion (after Bourne[10]); *Am* = amnion. (Methylene blue. ×500.)

gen and placed on silica gel TLC plates (Kieselgel 60 F_{254}, Merck, West Germany), with 20 μg each of cortisol and cortisone as carrier steroids. The plates were developed in chloroform/methanol/water (182/17/1, by volume). The plates were scanned for radioactivity with a Berthold LB 2723 Dünnsicht-Scanner II (Berthold, West Germany). The areas corresponding to cortisol and cortisone were visualized under ultraviolet light, scraped into scintillation vials, and eluted with ethanol. After the ethanol was evaporated, 10 ml of Fisofluor 2 (Fisons, Loughborough, England) was added and the radioactivity was measured in a Beckman LS 3133 T counter. ^{14}C-counts were used to correct each sample for recovery. Recoveries averaged 78.8% ± 0.7% (SEM, n = 56). 11β-HSD activity was calculated as the initial rate of conversion of substrate into product and was expressed as the percentage conversion. Identity of the metabolites was confirmed by recrystallization to constant specific activity.

Microscopy. Tissues from three sets of twins were fixed in 3% glutaraldehyde in 0.1M phosphate buffer. After postfixation in osmium tetroxide and dehydration in an ethanol series, the tissue was embedded in Araldite. Thick sections (1 μ) were cut on an ultramicrotome and stained with methylene blue for light microscopy.

Statistics. The Wilcoxon signed rank test was used for statistical comparisons. In each case results for choriodecidua and decidua were paired. The chorion was excluded since no 11β-HSD activity was found in this tissue. Samples from the first twin and the second twin were considered as independent pairs. Since it was expected from preliminary work that the values in the decidua would be higher than those in choriodecidua, the one-tailed test was used. All results are given as mean ± SEM.

Results

Scanning of the TLC plates revealed only two peaks corresponding to cortisol and cortisone. Cortisol:cortisone interconversion was, therefore, the only metabolism detected in the tissues.

Activity in the direction cortisol to cortisone (oxidative activity) was significantly higher in the decidua

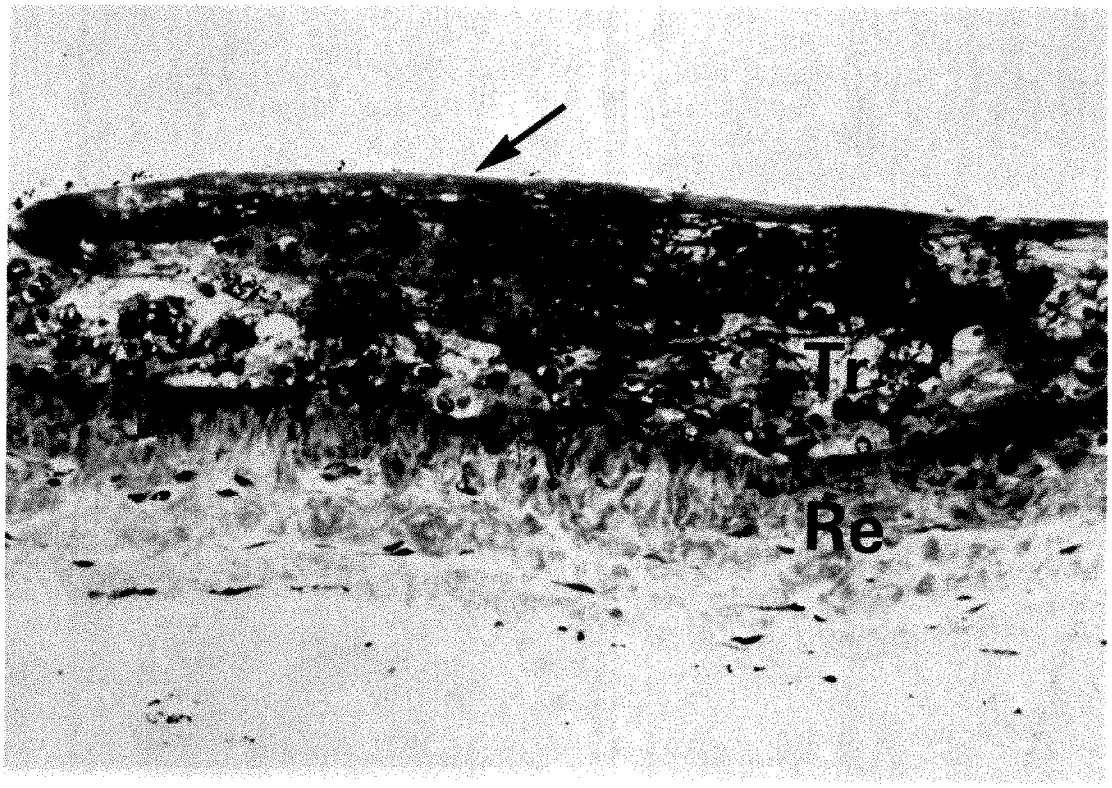

Fig. 4. Chorion obtained from the wall separating the two amniotic sacs in dichorionic twins. Note the lack of decidual tissue which is substituted by a fibrin-like material *(arrow)* which usually divides the two adjacent chorionic membranes. *Tr* and *Re* as in legend to Fig. 3. (Methylene blue. ×500.)

than in the choriodecidua: 53.4% ± 6.7% versus 38.5% ± 7.4% ($p < 0.05$, $n = 10$). No measurable 11β-HSD activity was found in the chorion ($n = 10$). Individual values are plotted in Fig. 1. Reductive activity (cortisone to cortisol conversion) was also significantly higher in the decidua than in the choriodecidua: 21.3% ± 2.2% versus 15.3% ± 3.2% ($p < 0.025$, $n = 10$). Again, no detectable activity was found in the chorion. Individual values are plotted in Fig. 2. In both decidua and choriodecidua oxidative activity was significantly higher than reductive activity ($p < 0.005$ for both).

Microscopic examination of the tissues confirmed the decidual contamination of the chorion detached from the uterine wall (choriodecidua, Fig. 3) and the "purity" of the chorion obtained from the wall separating the two amniotic sacs (chorion, Fig. 4).

Comment

It has been suggested previously[15] that decidua-free chorion from dichorionic twins might be used to determine whether prostaglandin production attributed to the chorion[16] is due to decidual contamination. Certainly for corticosteroid metabolism our preliminary findings[11] suggested that 11β-HSD activity might be located in the decidua rather than in the chorion as reported by others.[7, 13]

To the best of our knowledge, this is the first study where the question of decidual contamination of the chorion has been answered by measuring enzyme activity in membranes obtained from dichorionic twins. The results show that the chorion itself is devoid of 11β-HSD activity. Furthermore, decidual 11β-HSD activity is significantly higher than choriodecidual activity, in both oxidative and reductive directions. Since the same amount of tissue (as measured by wet weight) was used for all incubations, the lower activity found in the choriodecidua is probably due to the "inactive" chorionic fraction included in this tissue (Figs. 1 and 2). By means of histochemical methods, 11β-HSD activity has been described in the chorion (trophoblastic layer) in membranes obtained at term, which included one dichorionic twin pregnancy.[8] However, activity was described as "weak" and the methodology failed to show any 11β-HSD activity in the decidua. It could be argued that the chorion separating the two amniotic sacs may be degenerative since it receives no nutritive supply from underlying decidua, and this may influence its metabolic activity. However, microscopic examination of this tissue showed its structure to be similar to that of decidua-contaminated chorion (Figs. 3 and 4). Therefore, we conclude that 11β-HSD is of decidual, rather than chorionic, origin.

With our assay conditions, 11β-HSD activity in the decidua and choriodecidua is higher in the oxidative than in the reductive directions. This is in keeping with our previous findings[14] and in accord with the use of tissue homogenates*[17] but does not necessarily go against the concept that, toward term, "chorionic"[13] or "amniotic"[18] membrane preferentially converts cortisone to cortisol, therefore providing the fetus with an extra-adrenal source of cortisol in amniotic fluid.

The fetal membranes have been implicated in the onset of labor in man.[1, 2] These tissues cover a large area in intimate contact with the uterine decidua and could therefore participate in the control of uterine muscle physiology. Of great interest has been the demonstration that these tissues possess enzymes related to steroid and prostaglandin metabolism.[1-8, 16] However, the possibility that decidual contamination might be responsible for some of the enzyme activities attributed to these tissues has not been fully considered in the past. Whether fetal (amnion and chorion) or maternal (decidua) tissues are responsible for the metabolism of hormones involved in the control of parturition is of special relevance, particularly with regard to the hypothesis of a genetically controlled timing of the onset of labor.[15] The use of dichorionic twins, as in this study developed for 11β-HSD, could be applied to studies of other steroid- and prostaglandin-metabolizing enzymes.

We thank the staff of the delivery suite, John Radcliffe Hospital, Oxford, England, for their help in the collection of samples, and Miss Elaine Johnson for technical assistance. Dr. Patricia Yudkin was a statistical consultant. Professor L. Abad Martínez (Murcia, Spain) provided valuable comments.

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*López Bernal, A.: In preparation.

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Diagnosis of fetal death in utero with amniotic fluid creatine kinase

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There is a need for a widely available and accurate method for diagnosing fetal death in utero. The accepted standard technique for making this diagnosis is real-time ultrasonography, but this procedure is not available at all hospitals. However, almost every hospital laboratory has the capability of measuring creatine kinase in body fluids. One hundred eight pregnant women underwent real-time ultrasonography and analysis of amniotic fluid for creatine kinase. Ninety-one women with conditions that required amniocentesis during the second and third trimesters had a live fetus by ultrasonographic examination and a low level of amniotic fluid creatine kinase (0 to 3 sigma units per milliliter). Seventeen women with a dead fetus by ultrasonographic examination had an abnormally high level of amniotic fluid creatine kinase (5 to 9,800 sigma units per milliliter). The only erroneous diagnosis in this series involved a set of twins, one of whom was shown by real-time ultrasonography to be alive and the other dead. Both sacs contained abnormally high, although different, levels of creatine kinase. Analysis of creatine kinase in amniotic fluid is a reliable technique for diagnosing fetal death. (AM. J. OBSTET. GYNECOL. 138:1173, 1980.)

FETAL DEATH in utero is an emotionally difficult experience for both the gravid woman and her physician, and the situation may be exacerbated by the uncertainty which often surrounds the diagnosis. Various techniques have been used to diagnose fetal death. A history of absent fetal movement is subjective and prone to error, and may indicate fetal jeopardy rather than fetal death.[1] Auscultation of fetal heart sounds and fetal electrocardiography are more objective tests, but false negatives are well known to occur.[2-4] Although there are several radiographic signs of fetal death, such as collapse of the fetal skull and intrafetal gas, these require days to weeks to develop, and the use of radiographic procedures necessitates exposure of a potentially viable fetus to x-radiation. The use of Doppler ultrasound is a reasonably reliable means for determining the presence or absence of fetal life, but errors have occurred with this technique.[5] Ultrasound B-scanning has also been used,[5] but considerable expertise is required to interpret the scans.

The most sensitive and specific technique for diagnosing the presence or absence of fetal life is real-time ultrasound.[6, 7] Using this method, Levine and Filly[6] made only one error in 390 cases, that in a patient with twins, one of whom was dead. Since real-time sonography is not currently available at all hospitals, there is a need for a simple laboratory test which has a high predictive value for both fetal life and death.

In 1974, Kerenyi and Sarkozi[8] proposed that the measurement of creatine kinase (CK) in amniotic fluid may be a valuable test for determining the presence or absence of fetal life, but their work has apparently received little attention. The present study was undertaken to: (1) confirm the findings of Kerenyi and Sarkozi; (2) assess the effects of a variety of maternal and fetal conditions on CK activity in amniotic fluid; (3) study the effects of blood and meconium staining of amniotic fluid on CK activity; and (4) attempt to determine the source of increased CK activity in amniotic fluid after fetal death.

Material and methods

Patients. One hundred and eight pregnant women underwent real-time sonography and amniocentesis. One patient with twins, one alive and one dead, underwent amniocentesis on both sacs. The indications for amniocentesis, as well as associated maternal and fetal conditions, for the 92 patients who had a live fetus

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Received for publication June 3, 1980.

Accepted August 13, 1980.

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Table I. Indications for amniocentesis and associated conditions with a live fetus*

Indication	*No. of patients*
Repeat cesarean section	28
Hypertension	14
Diabetes mellitus	13
Premature labor	11
Isoimmunization	6
Hydramnios	5
Postaglandin-induced abortion	4
Twin gestation†	4
Possible amnionitis	3
Fetal neural tube defect	2
Postdates	2
Positive oxytocin challenge test	2
Premature rupture of membranes	2
Large-for-gestational age fetus	1
Chronic renal disease	1
Pneumonia	1
Breech presentation	1
Thanatophoric dwarf	1
Herpes genitalis	1
Sickle cell anemia	1
Placenta previa	1
Elective induction of labor	1
Third-trimester bleeding	1
Falling urinary estriol levels	1

*Nine patients had two indications; three patients had three indications.

†In one case of twins, one twin was dead.

by real-time sonography are listed in Table I. Fourteen patients were in the second trimester of pregnancy, and 78 patients were in the third trimester. Amniocentesis was performed in these patients only for diagnostic or therapeutic purposes (lecithin:sphingomyelin ratio, Δoptical density at 450 nm, instillation of abortifacients, etc.), and not solely for this study. Seventeen patients had a dead fetus as determined by real-time sonography, and all subsequently were delivered of a dead fetus. Amniocentesis was performed on these patients in order to instill abortifacients or to observe the color of the amniotic fluid. The cause of fetal death, when known, and the estimated length of time that the fetus had been dead at the time of amniocentesis are shown in Table II. Seven of these patients were in the second trimester and 10 were in the third trimester at the time of fetal death.

Real-time sonography. All patients underwent real-time sonography prior to amniocentesis. Scans were performed with a linear-array, real-time scanner using a 3.5 megahertz transducer (ADR model 2130, Tempe, Arizona). The diagnosis of fetal life was based upon the observation of fetal cardiac motion. Fetal death was diagnosed if fetal cardiac and vascular motions were absent. Frequently, other suggestive signs of fetal death, such as collapse of the fetal skull or scalp edema, were also observed.

Creatine kinase determination. Specimens of amniotic fluid were centrifuged at 3,000 rpm for 10 minutes and the supernatant was stored at 10° C. CK activity was determined within 24 hours of collection by the Rosalki method[9] and Sigma No. 45 UV reagents (Sigma Chemical Co., St. Louis, Missouri) at 25° C. The adult serum CK reference range is 0 to 12 sigma units per milliliter. CK isoenzymes were determined by electrophoresis on agarose with fluorescent densitometry by means of the Corning ACI method (Corning Medical, Medfield, Massachusetts).

Results

CK activity of amniotic fluid. Ninety-two patients with a live fetus determined by real-time sonography underwent amniocentesis. With the exception of the four patients who underwent prostaglandin-induced abortion, all subsequently were delivered of a live fetus. In 91 of the 92 cases, the amniotic fluid CK activity was 0 to 3 sigma units per milliliter. In the one other case, the CK activity was 210. In the latter case, the fetus was alive, but its monochorionic twin in the other sac was dead, with an amniotic fluid CK of 1,070. Possible explanations for this finding are discussed below. In 17 patients, a dead fetus was diagnosed by means of real-time ultrasonography, and all 17 were delivered of a dead fetus. The results of analysis of CK in amniotic fluid are shown in Table II. All 17 of these patients had an amniotic fluid CK activity of more than, or equal to, 5 sigma units per milliliter (range, 5 to 9,800). There was a rough positive relationship between CK activity and the length of time since fetal death (r = 0.39).

CK electrophoresis was performed in 11 cases of fetal death (Table II). In all cases the CK-MM isoenzyme was the predominant isoenzyme. Small amounts of CK-MB and CK-BB isoenzymes were found in some of the cases.

Color of amniotic fluid. The color of the amniotic fluid was noted in all 109 samples. The results are shown in Table III. Ten of the samples from living fetuses contained either bilirubinoid pigments, meconium, or blood, and all had CK activities of 0 or 1 sigma units per milliliter. One live fetus with a dead twin in the other sac had brown fluid. Most of the amniotic fluids from dead fetuses were brown; one fluid was normal colored, and two were yellow.

Comment

Wherever available, real-time ultrasonography is the method of choice for ascertaining the presence or ab-

Table II. Amniotic fluid creatine kinase in patients with dead fetus

No.	*Cause of death*	*Estimated time since fetal death (days)*	*CK total* (sigma units/ml)*	*CK-MB (sigma units/ml)*	*CK-BB (sigma units/ml)*
1.	Isoimmunization	1-2	5		
2.	Multiple anomalies	1-2	10	0.5	2.9
3.	Idiopathic hydrops	3	31		
4.	Anencephaly	2-3	41	Trace	ND
5.	Fetal cardiac anomaly	6	58		
6.	Fetomaternal hemorrhage	2	196	Trace	Trace
7.	Unknown	2-3	255	ND†	ND
8.	Unknown	4	316	Trace	Trace
9.	Unknown	4	420	ND	ND
10.	Unknown	14-21	420		
11.	Unknown	Unknown	790	Trace	Trace
12.	Unknown	28	880	ND	ND
13.	Unknown	21	930	ND	ND
14.	Twin-twin transfusion	21	1,070		
15.	Unknown	14-21	1,900		
16.	Unknown	56	3,900	Trace	ND
17.	Unknown	14	9,800	ND	ND

*Normal serum range, 0 to 12 sigma units per milliliter.
†ND = None detected.

Table III. Color of amniotic fluid

No.	*Color*	*Comment*	*CK (sigma units/ml)*
Live fetuses			
81	Normal		0-3
5	Yellow	Bilirubinoid pigments	0-1
3	Green	Meconium stained	0
2	Red	Blood stained	0-1
1	Light brown*		210
Dead fetuses			
1	Normal		196
2	Yellow	Bilirubinoid pigments	5,31
1	Red	Blood stained	10
13	Muddy brown		41-9,800

*Live twin with dead twin in other sac.

sence of fetal life.[6, 7] Since this technique is not available at all hospitals, a widely available and reliable test for fetal life is needed. Such a test is the measurement of creatine kinase in amniotic fluid, since this can be accomplished in almost all hospital laboratories. Kerenyi and Sarkozi[8] found low activities of CK in the amniotic fluid in 145 normal pregnancies, and increased activities in 12 cases of fetal death. CK activity was normal in one case in which the fetus had been dead for less than 2 days. Those authors also demonstrated a positive relationship between amniotic fluid CK activity and the length of time since fetal death, and stated that the level of CK in the amniotic fluid probably depended on factors other than the length of time since fetal death, such as fetal tissue mass and amniotic fluid volume.

There are few other references to amniotic fluid CK in the literature. Mahoney and associates[10] found the amniotic fluid CK activity to be normal at 16 weeks' gestation in a woman carrying a fetus with muscular dystrophy. Chaube and Swinyard[11] found increased amniotic fluid CK activities in trypan blue–treated rats with neural tube defects.

Our study has confirmed the findings of Kerenyi and Sarkozi. Amniotic fluid creatine kinase is a sensitive and specific indicator of fetal death. We found low activities of CK in the amniotic fluid of all cases of live fetuses, except one, and increased activities of CK in all cases of fetal death. The CK activity appeared to increase with the length of time that the fetus had been dead; but, on the basis of our data, this cannot be used reliably to determine the time since fetal death. Amniotic fluid CK activity was unaffected by a wide variety of maternal and fetal conditions, including chronic fetal distress and fetal neural tube defects. CK activity was also unaffected by meconium, blood, and bilirubinoid pigments in the amniotic fluid.

There was only one false result among the 109 samples. This case involved a monochorionic live twin with a dead twin in the other sac. It is unlikely that the CK molecule, with a molecular weight of 80,000, diffused across the dividing membrane. It is much more likely that the dividing membrane was damaged at the time of amniocentesis. It is interesting to note that the only error in Levine and Filly's study of real-time ultrasonography also involved a set of twins, one of whom was dead.[6] In the case of twins, abnormal amniotic fluid CK activity should be interpreted with caution.

The source of the CK activity in the amniotic fluid after fetal death is most likely the skeletal muscle of the fetus. After fetal death, amniotic fluid penetrates the fetal epidermis,[5] and skin slippage begins within a few hours.[12] Only a few tissues contain high activities of CK, and of these, only skeletal muscle contains the CK-MM isoenzyme almost exclusively.[13] The small amounts of CK-MB isoenzyme found in several samples may have come from skeletal muscle, and the trace amounts of CK-BB isoenzyme may have come from myometrium.

The color of amniotic fluid is often assumed to be a reliable indicator of fetal life or death. Jaffin and associates[14] found brown amniotic fluid in six cases of fetal death, and concluded that this finding may be relied on as absolutely confirmatory evidence of fetal death. Kerenyi and Sarkozi[8] observed that dark fluid may be found in the presence of fetal life, and normal-colored fluid may be found in association with fetal death. Golbus and associates[15] and Seller[16] both described dark brown or green-brown fluid at midtrimester amniocentesis in conjunction with normal fetuses, and attributed this color to aged blood pigments. Since we found normal-colored fluid in one instance of fetal death, and brown fluid in one case of a live fetus, we concluded that the color of amniotic fluid is not a reliable indicator of fetal life or death.

In summary, the analysis of creatine kinase in amniotic fluid is a readily accomplished and accurate means of diagnosing fetal death. Creatine kinase activity starts to rise within 2 days after fetal death, and fetal skeletal muscle is probably the source of the CK. The measurement of CK in amniotic fluid should be utilized in centers where real-time ultrasonography is not available. The color of amniotic fluid should not be relied on for the diagnosis of fetal life or death.

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Distribution of cardiac output in the unstressed pregnant guinea pig

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Cardiac output (CO) and organ flows were measured in five nonpregnant and 14 pregnant unstressed guinea pigs between the thirty-ninth and sixty-fifth postconceptional day by means of radionuclide-labeled microspheres. Blood pressure, heart rate, and cardiac output in nonpregnant animals were 60 torr, 234 bpm, and 248 ml/min, respectively. These parameters did not change significantly during pregnancy. The uterine fraction of cardiac output increased to ≈18% at term and there was an equivalent decrease in the CO fractions to the carcass and skin. Blood flow per gram of wet weight increased during pregnancy by approximately 140% and 70% for the thyroid and gastrointestinal tract, respectively, but did not change significantly for the heart, brain, kidneys, and adrenals. In pregnant animals near term the weight of the heart, kidneys, intestines, and lungs was approximately 25% lower than that observed in nonpregnant guinea pigs. (AM. J. OBSTET. GYNECOL. 138:1177, 1980.)

CARDIOVASCULAR CHANGES constitute an important part of the maternal adaptation to pregnancy. Information on this matter indicates marked species differences in both total cardiac output (CO) and its distribution. During primate[1, 2] and sheep[3] pregnancy CO increases by about 30% and 75%, respectively. Changes in CO distribution tend to be more pronounced when the CO fraction to the uterus in a particular species is larger. The uterine fraction of CO at term has been found to vary between 5% in the monkey[2] and 16% in the sheep,[3] whereas the size of the uterus plus conceptus in these species grows to 6% and 8% of maternal weight, respectively.

In the pregnant guinea pig the uterus plus conceptus may constitute as much as 30% of maternal weight,[4] which is considerably larger than in other small animals such as the rabbit (8%)[5] and rat (18%).[6] Furthermore, the relatively large fetus in the near-term guinea pig grows at a rate of 7% per day.[7] Therefore, it is likely that during late pregnancy major cardiovascular alterations are necessary in this species to accommodate the rapid growth of the already large fetus. The objective of the present study was to investigate the physiologic changes in maternal cardiovascular dynamics during the course of pregnancy in the guinea pig.

To avoid interference from anesthesia and surgical stress with normal cardiovascular[8-10] and metabolic[11] function, experiments were performed in conscious, unstressed animals.

Material and methods

Experiments were carried out in five nonpregnant and 14 pregnant albino guinea pigs ranging in weight from 813 to 965 and from 806 to 1,405 grams, respectively. All animals were obtained from the "T. N. O." Institution, Rijswijk, Holland. Pregnant animals with known postconceptional dates arrived in our laboratory shortly after conception. The animals were allowed at least 2½ weeks for adaptation to their new environment. The animals received food and water ad libitum until operation. In pregnant animals operation was performed before the fifty-fifth postconceptional day (term = 68 days) because of a high abortion rate associated with operation later on in pregnancy.

General anesthesia was induced with ketamine hydrochloride (2 mg · 100 g^{-1}, subcutaneously), xylazine (400 μg · 100 g^{-1}, intramuscularly) and atropine (5

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Received for publication June 3, 1980.

Accepted September 3, 1980.

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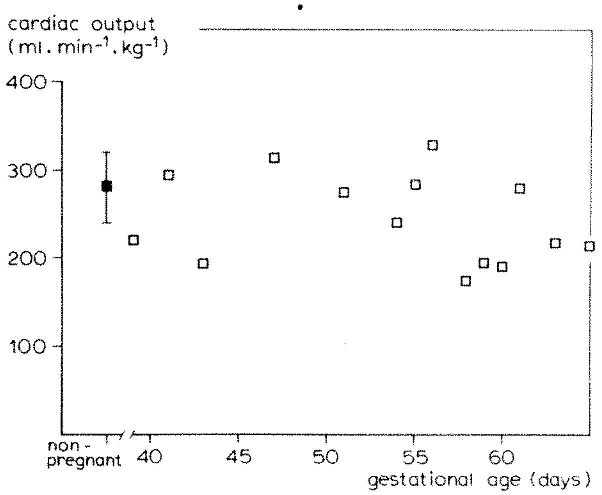

Fig. 1. CO during guinea pig pregnancy (□). The black square (■) indicates the mean ± SEM for the nonpregnant animals.

Table I. Hemodynamic measurements in guinea pig pregnancy

	Nonpregnant *(n = 5)*	*Pregnant*	
		39-56 days *(n = 8)*	*57-66 days* *(n = 6)*
HR (bpm)	234 ± 13	250 ± 22	230 ± 11
Mean systemic BP (torr)	60 ± 3	60 ± 3	58 ± 4
CO:			
ml · min^{-1}	248 ± 30	269 ± 21	231 ± 13
ml · min^{-1} · kg^{-1}	280 ± 38	276 ± 18	212 ± 18

Values are means ± SEM.

$\mu g \cdot 100\ g^{-1}$, intramuscularly). Adequate anesthesia was maintained throughout operation by adding nitrous oxide by mask as required. The animals were placed in the dorsal recumbent position.

By means of techniques described elsewhere,[12] polyethylene catheters of standardized caliber (outside and inside diameters = 0.80 and 0.40 mm, respectively) with a tapered and slightly curved tip were inserted into the left common carotid artery and a femoral artery and advanced into the left ventricle and abdominal aorta, respectively. The catheters were tunneled subcutaneously and exteriorized between the scapulae. Most animals recovered within 4 to 6 hours after operation. During this recovery period the animals were kept warm with an infrared light. Food intake was sharply reduced on the first day after operation but increased to preoperative quantities within 3 days. This phenomenon was considered to be an indication of complete fetal and maternal recovery since aborting and nonsurviving animals showed incomplete restoration of food intake. In order to minimize postoperative manipulation, the catheters were flushed once every other day with heparinized saline (1:400).

The CO distribution was determined between the fifth and fourteenth postoperative days. This time coincided in nonpregnant animals with diestrus and in pregnant animals with a range between the thirty-ninth and sixty-fifth postconceptional day. For the measurements, the animals were placed in a cardboard box of about four times their body size. Heart rate (HR, in beats per minute) and blood pressure (BP, in torr) in the abdominal aorta were recorded promptly.

Stable readings were usually obtained within 20 minutes and were assumed to indicate steady-state conditions. The CO distribution was determined by means of the microsphere technique.

Microsphere technique. Radioactive "carbonized" microspheres, labeled with Sr^{85}, with a diameter of 15 ± 2 μm (SD), and suspended in 10% dextran, were obtained from the supplier (3M Company, St. Paul, Minnesota). Preparation and handling of the microspheres have been described previously in detail.[13] The microsphere suspension was diluted aseptically with normal saline to a final concentration of 400,000 microspheres per milliliter. A sample of this suspension was placed in a preweighed glass injection vial and warmed to 39° C. Based on previous experience in fetal lambs[14] a total of 60,000 microspheres per 100 gm of maternal weight was injected (with constant mixing) into the left ventricle over a 60-second period. Starting from 30 seconds before and continuing until 30 seconds after the injection, a reference sample of blood was withdrawn at a constant rate (0.80 ml/min) from the abdominal aorta. This method of mechanical integration[15] permits blood flow calculation by the formula:

$$\text{Organ flow (ml/min)} = \frac{\text{number of microspheres in organ}}{\text{number of microspheres in arterial reference sample}} \times \text{withdrawal rate.}$$

The CO was calculated as the sum of all organ flows. Before and after the infusion of microspheres the position of the left ventricular catheter was verified with the pressure transducer. HR and BP in the abdominal aorta were recorded for 5 minutes after completion of the microsphere experiment. A blood sample was then collected from the abdominal aorta for measurement of blood gases, pH, hemoglobin, and hematocrit. Subsequently the animal was killed with an overdose of Pentothal; the catheter position was checked at autopsy, and all organs were dissected. On two occasions with suspicious pressure tracings, the tip of the left

Table II. Organ weights (grams) during guinea pig pregnancy

Organ	Nonpregnant (n = 5)	Pregnant	
		39-56 days (n = 8)	57-66 days (n = 6)
Total maternal mass	896 ± 31	972 ± 54	1,147 ± 61*
Total fetal mass	—	92 ± 22†	251 ± 23*
Uterus with contents	2 ± 1	118 ± 23†	294 ± 29*
Myometrium	2 ± 1	12 ± 1	20 ± 3
Mammary gland	6 ± 2	11 ± 1	9 ± 1
Carcass	484 ± 28	439 ± 17	456 ± 18
Skin	167 ± 9	166 ± 9	181 ± 19
Brain	4.4 ± 0.1	4.2 ± 0.1	4.0 ± 0.1
Heart	3.6 ± 0.4	2.5 ± 0.2†	2.6 ± 0.3†
Kidneys	8.8 ± 0.4	6.9 ± 0.4†	6.6 ± 0.3†
Gastrointestinal tract	43 ± 1	38 ± 1†	31 ± 1*
Pancreas	1.4 ± 0.4	1.7 ± 0.2	1.2 ± 0.1
Spleen	2.1 ± 0.5	1.5 ± 0.1	1.2 ± 0.1
Liver	38 ± 2	32 ± 2	36 ± 3
Lungs	10.1 ± 1.2	6.1 ± 1.0†	7.8 ± 0.9
Adrenals	0.90 ± 0.13	0.68 ± 0.04	0.82 ± 0.12
Thyroid	0.15 ± 0.02	0.13 ± 0.02	0.13 ± 0.02
Ovaries	0.40 ± 0.09	0.24 ± 0.02	0.22 ± 0.02

Values are means ± SEM.

*Significant change (unpaired t test, $p < 0.05$) as compared to early pregnancy (39 to 56 days).

†Significant change (unpaired t test, $p < 0.05$) as compared to the nonpregnant state.

ventricular catheter was located in the aortic valve. In these animals no myocardial blood flow could be calculated and CO was determined by adding an estimated myocardial flow. It was calculated that this estimation procedure could introduce a maximum error of less than 4%. The tip of the abdominal aorta catheter was always located between the level of the kidneys and the aortic bifurcation.

Organ dissection. First the skin was removed and weighed. After the dissection of both mammary glands, multiple skin aliquots were taken from standardized areas and placed into preweighed counting vials. Then each of the following organs was carefully dissected and placed in preweighed vials: thyroid, thymus, lungs, heart, brain, and intra-abdominal organs except for intestines and uterus. Paired organs were placed in separate vials. Lungs and liver were cut into suitable pieces and divided over several vials. The gastrointestinal tract was opened longitudinally over its entire length and the contents were rinsed out with normal saline. The cleaned gastrointestinal tissue was divided into four portions that were anatomically easy to recognize: stomach, small intestines, cecum, and colon. These pieces were reduced to suitable aliquots and also placed in preweighed counting vials. The uterus was dissected next. The placentas were divided into upper and lower portions, as described previously,[16] and placed in preweighed vials in the same order as their location within the uterus. The dissection of the uterine horns, cervix, and vagina was performed as described by Chaichareon and co-workers.[17] The remainder of the maternal body, referred to as the "carcass," and consisting primarily of skeletal muscle, bone, connective tissue, and fat, was ground with a Waring Blendor, and aliquots of the homogenate were placed in preweighed vials. The height of all tissue aliquots in the vials was kept below 1.0 cm to avoid a loss in counting efficiency.[13] Finally, the radioactivity in each aliquot and in the reference sample was determined with a well scintillation counter (Packard Autogamma Scintillation Counter 5220). For statistical reasons[18] the actual number of microspheres was determined in each sample from the counts per minute per microsphere and the counts per minute per sample. All reference samples contained at least 700 microspheres. In some animals the smallest organs, such as the thyroid and ovaries, contained less than 200 microspheres, giving rise to an increased statistical error in the related flow determination. Good mixing of the microspheres in the present study was suggested by the close correlation between the number of microspheres distributed to paired organs (kidneys and adrenals: $r = 0.95$ and 0.94 in nonpregnant animals [$n = 5$] and $r = 0.96$ and 0.92 in pregnant animals [$n = 14$]).

In the text, means are given together with their standard errors. When needed, the results were evaluated

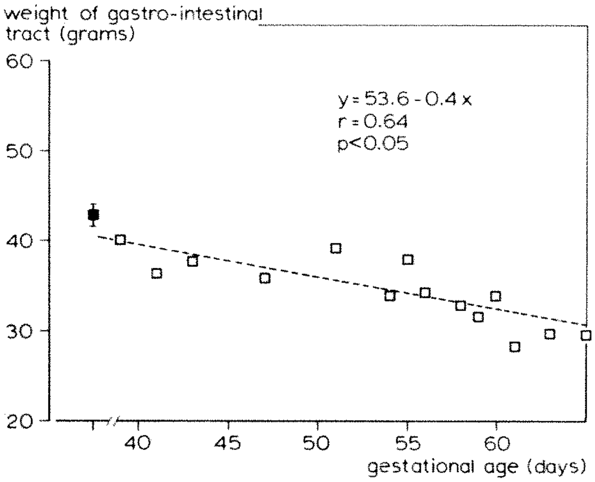

Fig. 2. Change in gastrointestinal weight in the course of guinea pig pregnancy (□). The black square (■) indicates the mean ± SEM for the nonpregnant animals.

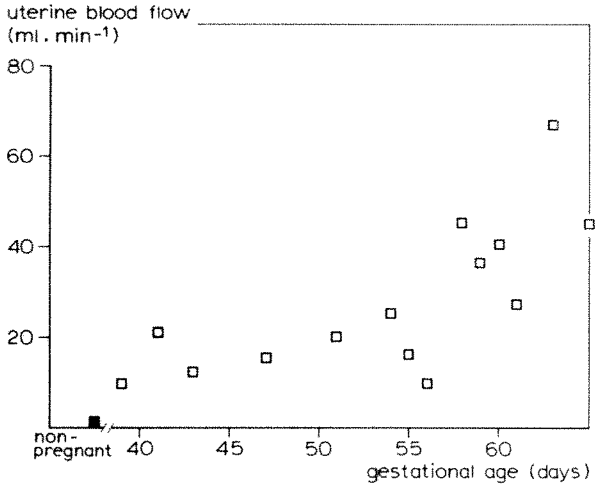

Fig. 3. Relationship between total uterine blood flow (ml · min⁻¹) and gestational age (□). The black square (■) indicates the mean uterine blood flow for the nonpregnant animals.

statistically by Student's t test (unpaired) or by linear regression analysis.

Results

The data for nonpregnant and two groups of pregnant animals on HR, mean BP, and CO are listed in Table I, whereas the pattern of CO per kilogram with postconceptional age is illustrated in Fig. 1. None of these parameters changed significantly during pregnancy. Changes in the organ weights, the fractional distribution of CO, and the regional blood flow are displayed in Tables II, III, and IV, respectively. The data in Table II show that the size of the uterus increased progressively during pregnancy. Furthermore, the weights of the heart, lungs, kidneys, and intestines in pregnant animals were 20% to 30% below nonpregnant values. Fig. 2 shows that during pregnancy the weight of the gastrointestinal tract decreased toward term. Data on the distribution of CO (Table III) demonstrate that in the last 10 days of pregnancy the rapidly increasing uterine fraction of CO was paralleled by a significant reduction in the CO fractions to the carcass and skin. Also the CO fractions to the kidneys and pancreas changed significantly during gestation.

Table III. Percent distribution of CO during guinea pig pregnancy

Organ	*Nonpregnant (n = 5)*	*Pregnant* 39-56 days (n = 8)	*Pregnant* 57-66 days (n = 6)
Uterus	0.5 ± 0.1	6.4 ± 0.8*	18.2 ± 1.8†
Mammary gland	0.7 ± 0.2	1.5 ± 0.2	1.4 ± 0.3
Carcass	46 ± 1	44 ± 4	34 ± 1*
Skin	11.5 ± 1.7	9.0 ± 0.9	5.4 ± 0.9*
Brain	2.1 ± 0.5	1.9 ± 0.2	1.7 ± 0.2
Heart	4.0 ± 0.5	3.0 ± 0.3	4.1 ± 0.9
Kidneys	14.3 ± 0.5	11.1 ± 1.3*	11.7 ± 1.0*
Gastrointestinal tract	16.4 ± 1.7	17.3 ± 1.4	20 ± 2
Pancreas	0.9 ± 0.2	2.3 ± 0.4*	1.4 ± 0.3
Spleen	1.7 ± 0.5	1.5 ± 0.3	1.3 ± 0.4
Liver‡	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
Lungs§	5 ± 1	7 ± 1	6 ± 1

Values are means ± SEM.

*Significant change (unpaired t test, $p < 0.05$) as compared to the nonpregnant state.

†Significant change (unpaired t test, $p < 0.05$) as compared to early pregnancy (39 to 56 days).

‡Hepatic artery flow.

§Fraction of injected microspheres recovered from the lungs (bronchial artery flow and systemic arteriovenous shunting).

Absolute and relative organ flows are listed in Table IV. During pregnancy the upper placental blood flow accounted for almost the entire rise in uterine blood flow. The almost unchanged absolute blood flow to the myometrium indicates that the marked increase in myometrial mass (Table II) was coupled with a corresponding decrease in flow per gram of tissue. Fig. 3 illustrates the progressive increase in UBF with gestation. This increase was due to an increase in PBF, which was closely correlated with the increase in total fetal weight (Fig. 4).

The most pronounced changes in nonreproductive tissue flow were observed in the carcass and skin, where absolute flow decreased in late pregnancy by more than

Table IV. Regional blood flow during guinea pig pregnancy

			Pregnant			
	Nonpregnant (n = 5)		*39-56 days (n = 8)*		*57-66 days (n = 6)*	
Organ	*ml · min⁻¹*	*ml · min⁻¹ 100 gm⁻¹*	*ml · min⁻¹*	*ml · min⁻¹ 100 gm⁻¹*	*ml · min⁻¹*	*ml · min⁻¹ 100 gm⁻¹*
Uterus:						
Myometrium	2.1 ± 0.8	80 ± 17	2.7 ± 0.5	20 ± 4*	2.4 ± 0.3	14 ± 3
Upper placenta	—	—	13 ± 2*	121 ± 16*	36 ± 4	224 ± 20†
Lower placenta	—	—	0.4 ± 0.1*	11 ± 2*	0.12 ± 0.02	5 ± 1†
Mammary gland	2.0 ± 0.6	36 ± 5	4.1 ± 0.7	41 ± 7	3.1 ± 1.0	33 ± 7
Carcass	115 ± 14	25 ± 4	120 ± 14	27 ± 4	77 ± 8†	18 ± 2†
Skin	29 ± 7	15 ± 2	24 ± 3	15 ± 1	13 ± 3†	7 ± 2†
Brain	4.9 ± 0.6	110 ± 13	5.2 ± 0.3	126 ± 10	4.3 ± 0.4	108 ± 10
Heart	9.9 ± 1.5	305 ± 46	7.9 ± 0.9	290 ± 58	7.8 ± 2.0	346 ± 104
Kidneys	36 ± 5	402 ± 51	28 ± 3	413 ± 46	29 ± 2	438 ± 34
Gastrointestinal tract:	39 ± 2	90 ± 5	47 ± 5	131 ± 11*	49 ± 2	156 ± 11†
Stomach	8.0 ± 1.1	131 ± 15	9.2 ± 1.0	155 ± 11	9.3 ± 0.9	216 ± 25*
Small intestine	15.6 ± 0.9	107 ± 12	16.1 ± 1.8	127 ± 16	18.0 ± 1.1	168 ± 14*
Cecum	7.2 ± 0.4	66 ± 4	11.2 ± 1.2*	134 ± 15*	11.4 ± 0.4*	167 ± 31*
Colon	8.2 ± 0.4	68 ± 5	10.0 ± 1.3	96 ± 10*	10.2 ± 0.6*	119 ± 11*
Pancreas	2.3 ± 0.6	182 ± 51	5.6 ± 1.4	337 ± 51	3.3 ± 0.7	285 ± 33
Spleen	4.6 ± 1.7	277 ± 18	4.1 ± 0.7	256 ± 29	3.7 ± 1.3	259 ± 77
Liver‡	1.3 ± 0.3	3.9 ± 0.6	0.7 ± 0.1	2.0 ± 0.2	0.9 ± 0.1	2.7 ± 0.3
Adrenals	0.74 ± 0.08	90 ± 12	0.60 ± 0.06	87 ± 7	0.52 ± 0.04	77 ± 9
Thyroid	0.17 ± 0.03	90 ± 18	0.24 ± 0.05	146 ± 31	0.23 ± 0.06	217 ± 41
Ovaries	0.31 ± 0.05	75 ± 14	0.54 ± 0.06*	240 ± 29*	0.30 ± 0.07	141 ± 28

Values are means ± SEM.

*Significant change (unpaired t test, $p < 0.05$) as compared to the nonpregnant state.

†Significant change (unpaired t test, $p < 0.05$) as compared to early pregnancy (39 to 56 days).

‡Hepatic artery flow.

30% as compared to early pregnancy (Table IV). Changes in blood flow to other nonreproductive tissues were, in general, small. Only the perfusion of the gastrointestinal tract increased significantly during pregnancy. However, while the flow per gram of tissue increased by 70%, absolute blood flow increased by only 26% because of the lower tissue weight during pregnancy. Blood flow increases in different regions of the gastrointestinal tract were largest in the cecum and smallest in the stomach.

Comment

These observations are probably the first reported on CO distribution in unstressed pregnant guinea pigs. Blood flows in this study were determined by means of the microsphere technique. Although this method has been used in pregnant and nonpregnant guinea pigs for several years, its validity in this species has never been explored. In the present study it was found in nonpregnant animals that 5% of the injected microspheres were recovered from the lungs, which is a lower amount than that which has been reported for microspheres of similar size in unanesthetized rabbits.[19] In a preliminary study in two pregnant guinea pigs, a negligible number of microspheres was collected in a blood sample withdrawn simultaneously with the arterial reference sample from the upper portion of the inferior vena cava. Since the fraction of injected microspheres in the lungs also did not increase during pregnancy, it was concluded that 15 μm microspheres are not shunted in significant amounts across the hemochorial placenta of the conscious guinea pig. The microsphere injection as performed in the present study neither gave rise to any noticeable maternal discomfort nor induced any appreciable change in HR and BP. However, experiments carried out in preparation for this study indicated that injecting a dose about three times as large as in the present study or infusing the suspension at twice the speed we employed could give rise to a transient rise in maternal BP and, occasionally, nystagmus. These effects have been previously described for the rabbit[20] and dog.[21] The injection of an ice-cold microsphere suspension may induce shivering. Finally, adequate mixing of microspheres at the injection site was suggested by the close correlation between the number of microspheres recovered from paired organs.

The data on HR and BP in the nonpregnant animal

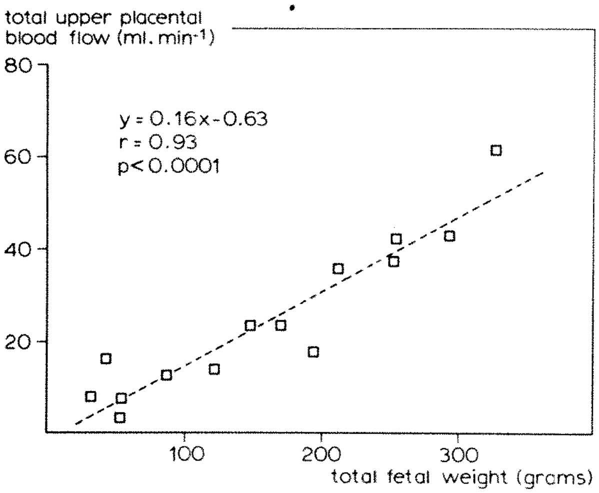

Fig. 4. Relationship between total upper placental blood flow and total weight of the conceptus.

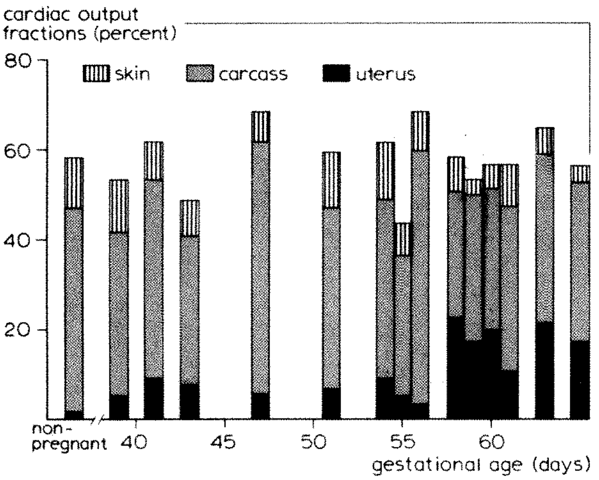

Fig. 5. The relationship of blood flow between the uterus, on the one hand, and the carcass and skin, on the other hand, in the course of guinea pig pregnancy.

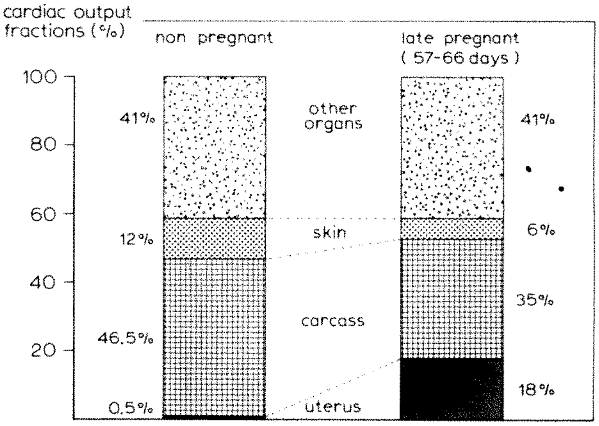

Fig. 6. The redistribution of CO during pregnancy in the guinea pig.

obtained in the present study were similar to those reported previously for unrestrained guinea pigs.[22, 23] Neither parameter changed significantly in the course of pregnancy. In several species mean arterial BP tends to decrease, and HR to increase, during pregnancy. The CO of 280 ml/min in the nonpregnant guinea pig was similar to that reported for the rat.[24] As expected from the relationship between CO and body weight,[25] the CO of the guinea pig per kilogram was twice as high as that of the rabbit[26] and monkey [2] and more than three times as high as that found in man[27] and sheep.[3] In contrast with the sheep[3] and the human,[1] the guinea pig does not experience an increase in CO during pregnancy. Apparently all circulatory demands in the pregnant state are effectively met by a redistribution of CO. Perhaps this is related to the fact that the CO of small mammals is already quite high in the nonpregnant state.

A marked increase in the uterine fraction of CO to as much as 20% at term dominated the redistribution of CO during guinea pig pregnancy. In other mammals this percentage has been found to vary between 5% and 18%, the fraction being generally in proportion to the term fetal-maternal weight ratio.[3, 5, 6, 28] Therefore, the higher CO fraction to the uterus in the near-term guinea pig is probably related to the higher fetal-maternal weight ratio in this species. Because of the correlation between placental blood flow and total weight of the conceptus (Fig. 4), the placental blood flow per unit of fetal weight was almost constant between the fifty-fifth and sixty-fifth postconceptional day. In contrast, Bjellin and co-workers[16] reported a gradual decrease of placental blood flow per kilogram of fetal weight with gestation. This observation might be related to the acute setup of their study. A detailed discussion on regional blood flow in the pregnant uterus of the guinea pig will be presented in a separate report.

Blood flow to the mammary gland was found to increase in guinea pig pregnancy although the increase was less striking than the one reported for the sheep.[29] The rise in mammary flow was proportional to the weight increase, indicating no change in relative flow. Although ovarian blood flow was significantly increased during pregnancy, the increase varied markedly and seemed related to the number of conceptuses in the ipsilateral horn, as reported by Bjellin and coworkers.[16] The reduction in ovarian blood flow in late pregnancy might be related to the increasing endocrine role of the placenta with advancing gestation.

In the last 10 days of pregnancy, changes in nonreproductive tissue flow were dominated by decreases in CO fractions to the carcass and skin, which seemed to be associated with the rapidly increasing uterine fraction of CO.

The resulting decrease in absolute flow was particularly impressive in the large vascular bed of the carcass (Fig. 5). Since BP remained unchanged, lower blood flows in the carcass and skin were a result of an increase in vascular resistance. It is not clear whether this increase in vascular resistance is due to neural, endocrine, or metabolic factors. The pregnancy-induced CO redistribution in the guinea pig resembles that in the near-term ewe,[3] suggesting that similar mechanisms may be operating in these species. Since epinephrine infusion in the near-term ewe causes vasoconstriction in the uterus and a marked vasodilatation in the carcass,[30] it is possible that the redistribution of CO in the pregnant ewe is triggered by a change in adrenergic receptor sensitivity in certain areas of the cardiovascular system. A change in the affinity of receptor binding sites for epinephrine and/or norepinephrine induced by some endocrine factor(s) of placental origin might also explain the CO redistribution. This hypothesis needs to be tested in further experiments.

In the present study the renal fraction of CO decreased significantly during pregnancy. The fall in absolute flow was not significant, because of the reduction in organ weight. A reduction in CO fraction to the kidneys has also been observed in the sheep. This phenomenon might have been triggered by a mechanism similar to the one which caused the increased vascular resistance in the carcass and skin.

The guinea pig fetus grows at a rate of 7% a day in the last 2 weeks of pregnancy.[7] This rapid growth rate gives rise to a variety of metabolic changes.[11] Food intake increases markedly. A steady increase in gastrointestinal blood flow during the course of pregnancy may be related to this increased nutritional demand. Whether the concomitant fall in intestinal weight could also be attributed to the increased metabolic demands of pregnancy is not clear. Weight reduction of other visceral organs seems to suggest a systemic rather than a local effect. Further investigation, such as measurement of wet weight/dry weight ratios and possibly tissue composition, is needed to solve this question.

The cardiovascular adaptation in the pregnant guinea pig is summarized in Fig. 6. The redistribution of CO was characterized mainly by a shift of flow from the carcass to the pregnant uterus. Flow changes in the visceral organs were small and probably related to a variety of metabolic changes necessary to accommodate the rapid growth of the relatively large fetus.

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SEMINARS IN OBSTETRICS AND GYNECOLOGY

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GYN CME FUND

GYNECOLOGY CONTINUING MEDICAL EDUCATION

JANUARY 31, 1981

CONTRACEPTION from PUBERTY to MENOPAUSE

9:00 a.m. - 9:45 a.m. Daniel R. Mishell, Jr., M.D.
"Oral Contraceptives"
10:00 a.m. - 10:45 a.m. Richard Marrs, M.D.
"Methods Of Abortion"
11:00 a.m. - 11:45 a.m. Daniel R. Mishell, Jr., M.D.
"I.U.D."
1:30 p.m. - 2:15 p.m. Richard Marrs, M.D.
"Sterilization Techniques"
2:30 p.m. - 3:15 p.m. Richard Marrs, M.D.
"Reversal Of Sterilization"
3:30 p.m. - 4:15 p.m. Daniel R. Mishell, Jr., M.D.
"Hormonal Treatment in the Menopausal Women"

FEBRUARY 14, 1981

PERINATAL INFECTIOUS DISEASE

8:00 a.m. - 8:45 a.m. Gilles R.G. Monif, M.D.
"Toxoplasmosis In Pregnancy"
9:00 a.m. - 9:45 a.m. Robert E. Harris, M.D.
"Urinary Tract Infections and Pregnancy"
10:00 a.m. - 10:45 a.m. William H. Swartz, M.D.
"Applicability of Prophylactic Antibiotics to Perinatology"
11:00 a.m. - 11:45 a.m. Gilles R.G. Monif, M.D.
"Herpetic Vulvovaginitis and Pregnancy"
1:30 p.m. - 2:15 p.m. Robert E. Harris, M.D.
"Syphilis In Pregnancy"
2:30 p.m. - 3:15 p.m. William H. Swartz, M.D.
"The Group B Beta Hemolytic Streptococci"
3:30 p.m. - 4:15 p.m. Gilles R.G. Monif, M.D.
"Rubella and Rubella Vaccine"
4:30 p.m. - 5:00 p.m. Panel Discussion

MAY 2, 1981

PEDIATRIC-ADOLESCENT GYNECOLOGY

9:00 a.m. - 9:45 a.m. W. Donald Woodard, M.D.
"Evaluation of Vaginal Bleeding in Early Childhood"
10:00 a.m. - 10:45 a.m. Stanley F. Gould, M.D., Ph.D.
"Structural-Congential Abnormalities of the Genito-Urinary System"
11:00 a.m. - 11:45 a.m. Bruce Albrecht, M.D.
"Dysmenorrhea in the Young Adolescent"
1:30 p.m. - 2:15 p.m. George Betz, M.D., Ph.D.
"Primary Amenorrhea"
2:30 p.m. - 3:15 p.m. Richard Z. Belch, M.D., Ph.D.
"Ovarian Neoplasms In Children"
3:30 p.m. - 4:15 p.m. Panel Discussion
"Evaluation of DES Problems in Infancy and Childhood"

JUNE 6, 1981

UPDATE: THE ENDOMETRIUM AND ENDOMETRIOSIS

9:00 a.m. - 9:45 a.m. Ralph M. Wynn, M.D.
"Histology and Ultrastructure of the Endometrium"
10:00 a.m. - 10:45 a.m. Ralph M. Wynn, M.D.
"Histogenesis and Pathology of Endometriosis"
11:00 a.m. - 11:45 a.m. Ralph M. Wynn, M.D.
"Miscellaneous Lesions of the Endometrium"
1:30 p.m. - 2:15 p.m. L.R. Malinak, M.D.
"Concepts of Treatment in Endometriosis: The Role of Medical and Surgical Therapy"
2:30 p.m. - 3:15 p.m. L.R. Malinak, M.D.
"Conservative Operations for Pelvic Endometriosis"
3:30 p.m. - 4:15 p.m. L.R. Malinak, M.D.
"Management of Advanced Endometriosis"

JULY 18, 1981

UPDATE: PERINATAL PROBLEMS

9:00 a.m. - 9:45 a.m. Louis Gluck, M.D.
"Fetal Lung Maturation"
10:00 a.m. - 10:45 a.m. Robert K. Creasy, M.D.
"Evaluation of Drugs to Stop Labor"
11:00 a.m. - 11:45 a.m. Louis Gluck, M.D.
"New Venereal Diseases"
1:30 p.m. - 2:15 p.m. Robert K. Creasy, M.D.
"Induction of Labor, 1981"
2:30 p.m. - 3:15 p.m. Louis Gluck, M.D.
"Asphyxia & Intraventricular Hemorrhage"
3:30 p.m. - 4:15 p.m. Robert K. Creasy, M.D.
"Intrauterine Growth Retardation"

AUGUST 15, 1981

PERILS AND PEARLS OF INFERTILITY INVESTIGATION AND TREATMENT

9:00 a.m. - 9:45 a.m. W.R. Keye, M.D.
"Avoiding Iatrogenic Infertility"
10:00 a.m. - 10:45 a.m. A. Marsh Poulson, Jr., M.D.
"Management of Ectopic Pregnancy"
11:00 a.m. - 11:45 a.m. William R. Keye, M.D.
"Unexplained Infertility"
1:30 p.m. - 2:15 p.m. A. Marsh Poulson, Jr., M.D.
"Microsurgical Aspects of Tubal Surgery"
2:30 p.m. - 3:15 p.m. William R. Keye, M.D.
"Psycho-Social Aspects of Infertility"
3:30 p.m. - 4:00 p.m. A. Marsh Poulson, Jr., M.D.
"Invitro Fertilization"
4:00 p.m. - 4:15 p.m. QUESTIONS & ANSWERS

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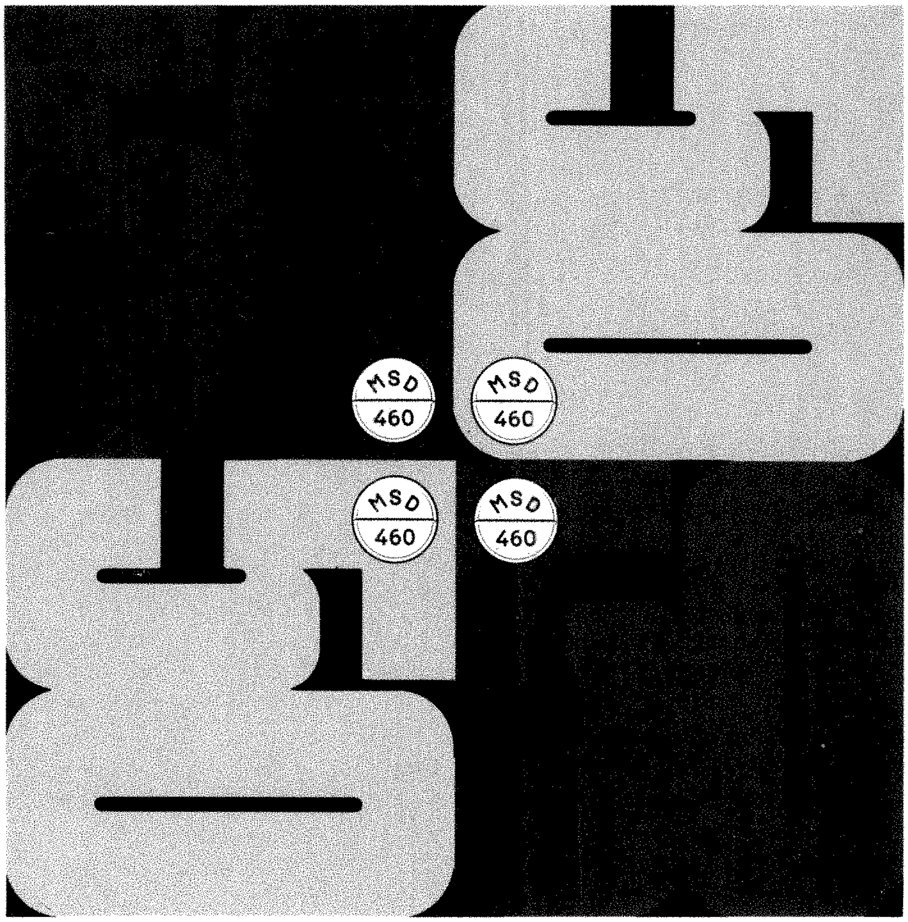

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Relation of maternal cigarette smoking, obesity, and energy consumption to infant size

J. C. HAWORTH
JUDITH J. ELLESTAD-SAYED
JEAN KING
LOUISE A. DILLING
Winnipeg, Manitoba, Canada

The dietary energy intakes of 153 public patients (94 smokers and 59 nonsmokers) and 383 Private patients (208 smokers and 175 nonsmokers) were assessed in the last month of pregnancy. Birth weight and crown-heel length of offspring were related to maternal size (weight for height) and smoking habits. Birth weight and length increased significantly with increasing maternal weight for height in the Private group but not in the Public group. In both groups, and in all weight categories, infants of smokers were lighter and shorter than those of nonsmokers. Neither the fetal growth retardation in the smokers nor the fetal growth enhancement in the overweight mothers was explainable on the basis of maternal dietary energy intake. Maternal obesity and cigarette smoking act independently of each other and maternal overweight does not protect the fetus against the growth-retarding effect of smoking. (AM. J. OBSTET. GYNECOL. 138:1185, 1980.)

INTRAUTERINE GROWTH RETARDATION of infants born to women who smoke cigarettes during pregnancy has been well documented.[1] The cause is not known but maternal undernutrition has been suggested.[2-4] In a series of 44,000 pregnancies, Garn and associates[4] found that the birth weights of infants of obese smokers were greater than those of infants of smokers in general and similar to those of infants of nonsmokers. They suggested that maternal obesity counteracted the growth-retarding effect of smoking on the fetus. Unfortunately, birth weights of obese nonsmokers were not included for comparison.

We[5] recently reported daily nutrient intakes of 302 smoking and 234 nonsmoking mothers. The dietary intake of energy and some nutrients of smokers slightly exceeded that of nonsmokers. The extra food consumed by the smokers was entirely accounted for by increased eating between meals.

We report here the relationship of infant size to maternal cigarette smoking, weight, and dietary energy consumption in the same group of subjects.

Subjects and methods

As previously reported,[5] we studied 153 Public patients (94 smokers and 59 nonsmokers) who attended the Hospital's antenatal clinic and 383 Private patients (208 smokers and 175 nonsmokers) who received their antenatal care in obstetricians' offices. Women with conditions that might have affected dietary intake or

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Study was supported by grants from the Mrs. James A. Richardson Foundation, the Manitoba Medical Services Foundation, and the Children's Hospital of Winnipeg Research Foundation.

Received for publication July 28, 1980.

Revised September 5, 1980.

Accepted September 9, 1980.

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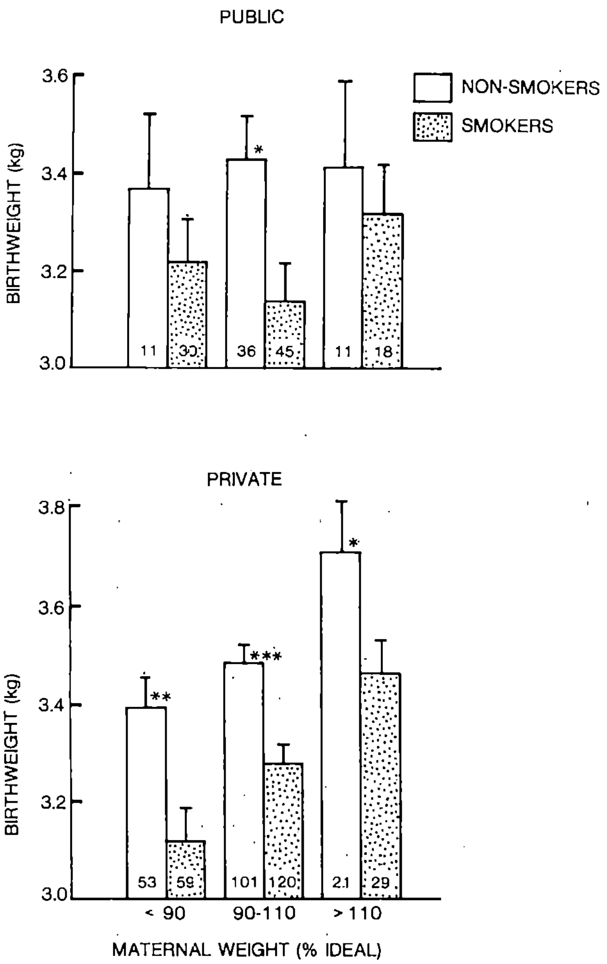

Fig. 1. Birth weight (mean ± 1 SEM) in infants of nonsmoking and smoking mothers in three maternal weight categories. Public and Private patients are shown separately. Numbers within the columns indicate the number of subjects. Asterisks indicate significant differences in the smoking and nonsmoking means for each pair of bars (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

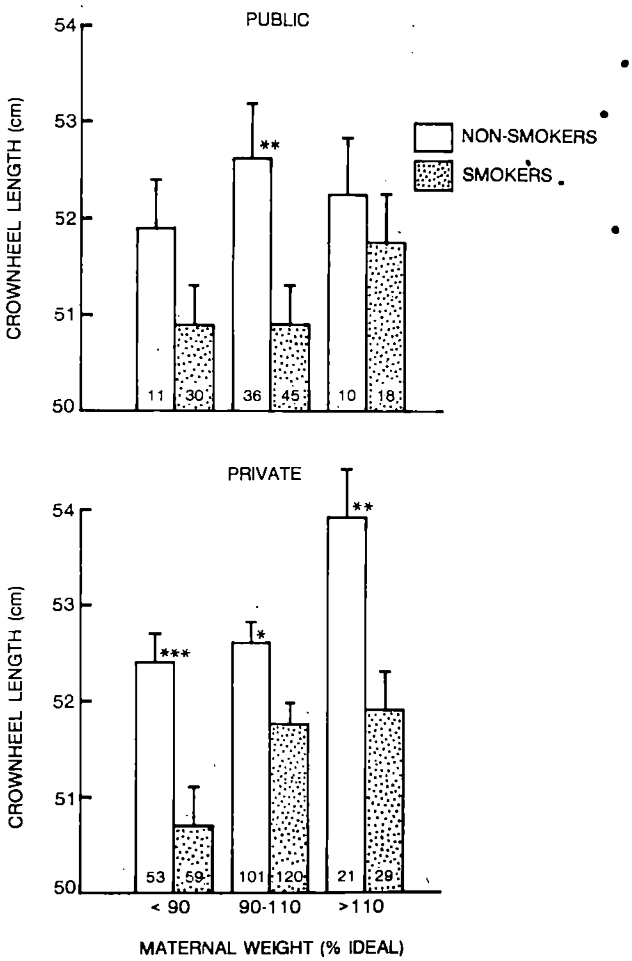

Fig. 2. Crown-heel length (mean ± 1 SEM) expressed as in Fig. 1. Asterisks same as in Fig. 1.

fetal growth were excluded (e.g., diabetes, renal disease, multiple pregnancy).

With informed consent, dietary intake was assessed in the last month of pregnancy as previously described. Women smoking one or more cigarettes a day were classified as smokers; the remainder, as nonsmokers. Height and prepregnancy weight were obtained at the first prenatal visit. From height and weight tables,[6] the mothers were divided into three weight categories: underweight (<90% ideal weight for height), normal weight (90% to 110%), and overweight (>110%). The infants were weighed and measured at birth. Data were computerized and analyzed by standard statistical methods.

Results

Public and Private patients were analyzed separately since maternal variables were very different in the two groups (Table I). There was no difference in any of the maternal variables between smokers and nonsmokers in the Public group. Private smokers were similar to nonsmokers in height, weight, and parity but smokers were slightly younger and had slightly less education and a smaller family income. In both Public and Private groups, infants of smokers had significantly lower birth weights (216 and 224 gm), were significantly shorter (1.2 and 1.3 cm), and had smaller head circumferences (0.3 and 0.4 cm, respectively). The sex distribution and the gestational age of infants of smokers and nonsmokers was not different in either group.

Fig. 1 shows the birth weights of infants of mothers in the three weight categories. Birth weight increased significantly with increasing maternal weight for height

Table I. Comparison of maternal variables (mean ± SEM) in Public and Private patients

Variable	*Public*	*Private*	*p*
No.	153	383	
Age (yr)	21.6 ± 0.4	25.6 ± 0.2	<0.01
Parity	1.04 ± 0.11	0.81 ± 0.04	<0.05
Height (cm)	160.4 ± 0.5	163.1 ± 0.03	<0.001
Prepregnancy weight (kg)	56.8 ± 0.8	57.0 ± 0.5	NS
Pregnancy weight gain (kg)	12.0 ± 0.4	13.3 ± 0.2	<0.01
Education (yr)	8.8 ± 0.2	12.1 ± 0.1	<0.001
Annual family income ($ × 10^3)	6.5 ± 0.1	11.3 ± 0.3	<0.001
American Indian (%)	61	1	<0.0001
Unmarried (%)	67	6	<0.0001

NS: Not significant.

in smokers and nonsmokers in the Private group ($p < 0.001$) but not in the Public group. In both groups, within each weight category, infants of smokers weighed less than infants of nonsmokers ($p < 0.001$) and the difference was of about the same magnitude in all categories, averaging 6% or 210 gm. When birth weights were controlled for gestational age and sex, this effect of smoking remained constant.

Crown-heel length (Fig. 2) did not differ by maternal weight category in the Public group but infants of Private overweight mothers were significantly longer than those of mothers in the other weight categories ($p < 0.01$). Infants of smokers were shorter than those of nonsmokers ($p < 0.001$) by an amount that was similar in each maternal weight category (average 1.3 cm or 2.5%).

Dietary energy intake in the Public group did not differ significantly by weight category (Fig. 3), but in the Private group, energy consumption decreased with increasing weight for height ($p < 0.01$). Energy consumption did not differ by smoking category except that Private smokers of normal weight ate more than nonsmokers ($p < 0.005$).

Neither birth weight nor crown-heel length differed by maternal energy intake in either the Public or Private group (Figs. 4 and 5) but the growth-retarding effect of smoking was approximately the same for all levels of dietary intake.

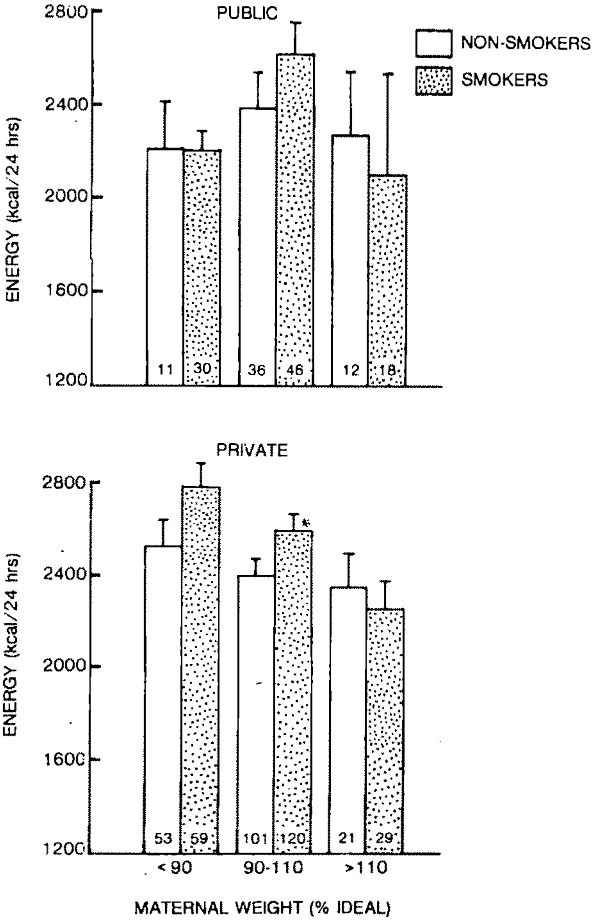

Fig. 3. Maternal energy intake (mean ± 1 SEM) expressed as in Fig. 1. Asterisks same as in Fig. 1.

Comment

This study confirms the findings of Garn and associates[4] that birth weights of offspring of overweight smokers are greater than those of smokers in general and similar to birth weights of nonsmokers. However, smoking did have a fetal growth-retarding effect in overweight mothers; the degree of retardation, in terms of weight and length, was approximately the same as that in infants of normal and underweight mothers. Obesity does not, therefore, counteract the effect of smoking, as suggested by Garn and associates.[4]

A simple nutritional cause for growth retardation in infants of smokers is unlikely because smokers eat the same amount, or even more, than nonsmokers.[5, 7-9] It is more likely that the growth retardation is due to a direct toxic effect of smoking on the fetus.[10]

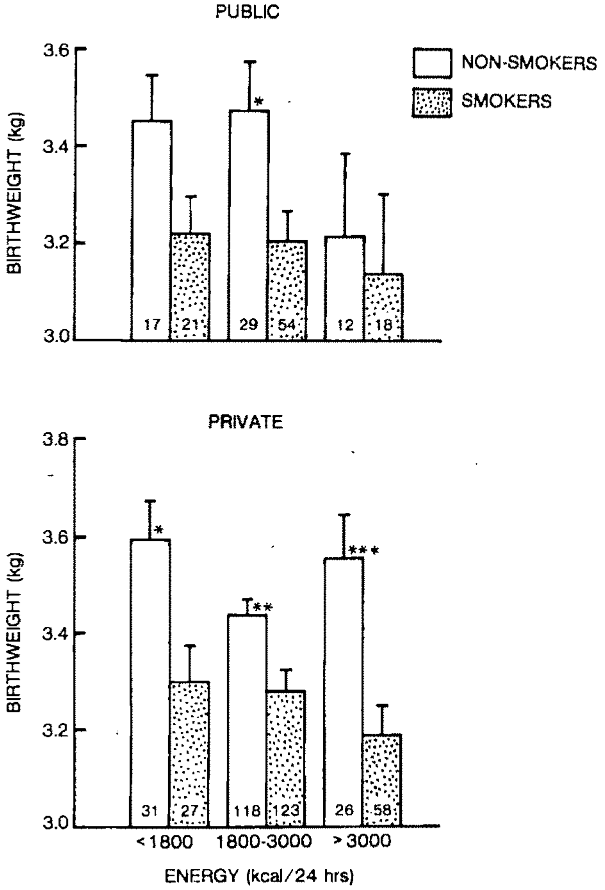

Fig. 4. Birth weight (mean ± 1 SEM) in infants of nonsmoking and smoking mothers in three maternal energy intake categories. Public and Private patients are shown separately. Numbers within the columns indicate the numbers of patients. Asterisks same as in Fig. 1.

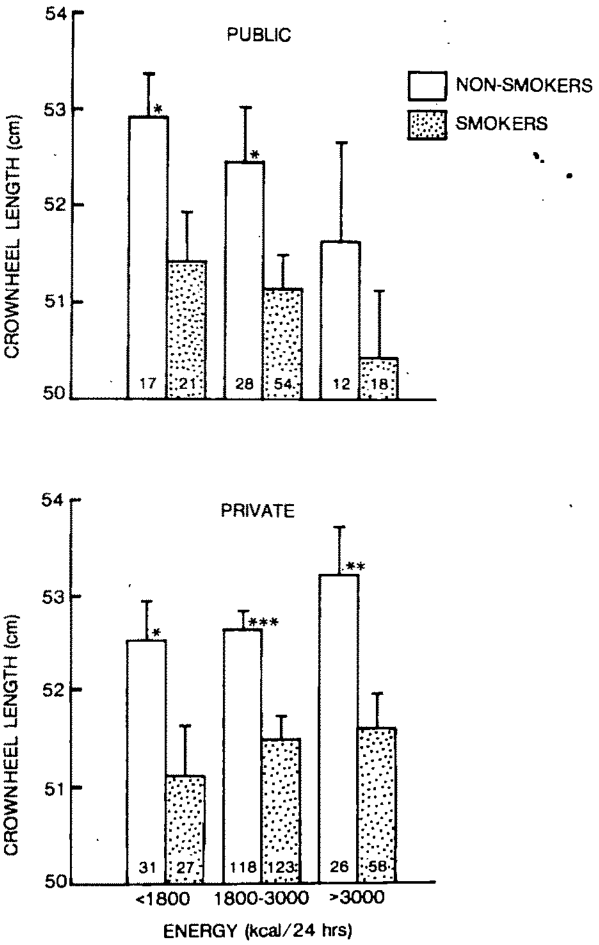

Fig. 5. Crown-heel length (mean ± 1 SEM) expressed as in Fig. 4. Asterisks same as in Fig. 1.

The increased size of infants born to overweight women in the Private group, as compared to normal or underweight women, was not explainable on the basis of increased energy consumption. The overweight patients in fact consumed significantly less food than their nonoverweight counterparts, confirming the findings of Beal[11] that pregnant overweight women tend to decrease their food intake. There was no correlation between birth weight and maternal energy intake.[5] The influence of maternal size on birth weight is well known[12, 13] and appears to be independent of pregnancy weight gain[14, 15] as well as maternal energy intake.

We conclude that the factors responsible for growth retardation in infants of smoking mothers and the factors involved in the growth enhancement of infants of obese mothers act independently of one another and neither effect is explainable on the basis of maternal dietary intake. Infants of overweight smokers have about the same degree of fetal growth retardation as infants of smokers in general.

We acknowledge with thanks the help of many individuals, including the staff of the Women's Centre and Mr. Fred Van Aertselaer for expert computer assistance.

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The effect of fetal heart rate on cardiovascular function during hypoxemia

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BENJAMIN T. JACKSON, M.D.*
Boston, Massachusetts

The effect of heart rate changes on cardiovascular function during hypoxemia was studied in lamb fetuses. In chronic preparations, we determined fetal heart rate, descending aortic blood flow (by electromagnetic flowmeter), Po_2, Pco_2, and pH and calculated fetal cardiac output (CO) and organ blood flows (with the use of 15 μ nuclide-labeled microspheres). Observations were made during control and hypoxemic states either alone or in the presence of parasympathetic blockade by atropine. Fetuses exposed to moderate hypoxemia (ewe breathing 10% oxygen) developed a significant bradycardia which was prevented by atropine pretreatment; no significant change in CO occurred in either case. Fetuses exposed to severe hypoxemia (8% oxygen) developed bradycardia and a significant decrease in CO; atropine pretreatment prevented the bradycardia but failed to prevent the decrease in CO. Atropine administered to the already severely hypoxemic fetus increased the heart rate but did not restore the depressed CO. The redistribution of blood flow from low-priority (e.g., viscera) to high-priority (e.g., myocardium) tissues correlated with the degree of hypoxemia. Myocardial blood flow was greater during hypoxemia when bradycardia was prevented by atropine than during hypoxemia alone; otherwise, blood flow distribution in response to hypoxemia was unaffected by atropine blockade. (AM. J. OBSTET. GYNECOL. 138:1190, 1980.)

THE FETAL RESPONSE to interference with its oxygen supply is a complex set of cardiovascular adjustments in which the heart rate slows, the arterial pressure increases, and a redistribution of blood flow occurs with increasing flow to the "higher priority" organs such as the brain and heart at the expense of "lower priority" organs such as the viscera and musculoskeletal tissues. This phenomenon has been studied extensively, in a qualitative sense[1, 2]; however, quantitative changes in overall fetal cardiac output and blood flows to all fetal tissues have been determined only relatively recently.[3, 4]

Rudolph and Heymann,[5] in studies on the relationship of the fetal heart rate to cardiac output in chronic fetal preparations under normoxic conditions, found that a linear relationship existed between fetal heart rate and ventricular output and that spontaneous falls in heart rate were associated with reductions in both right and left ventricular outputs. If this relationship also occurs during hypoxemia, then the slowing in heart rate seen as part of the fetal response might cause some decrease in cardiac output during hypoxemia; maintaining or elevating the fetal heart rate during hypoxemia might then lead to maintenance or augmentation of fetal cardiac output under such conditions. In 1974, we found that atropine blockade prior to fetal hypoxemia prevented the bradycardia usually seen but failed to prevent a significant decrease in cardiac output[6]; however, others have found subsequently that atropine during fetal hypoxemia not only increased the fetal heart rate but also augmented individual ventricular outputs.[7] The controversy that has arisen over the possible beneficial effect of atropine during fetal hypoxemia is understandable in view of the differences in methodology, definitions of hypoxemia, and levels of hypoxemia used by the various investigators. We undertook the present study to investigate specifically the role of fetal heart rate changes in the response to hypoxemia and the relative influence of

From the Laboratory for Perinatal Research, Department of Surgery, Boston University School of Medicine, and the Boston Veterans Administration Medical Center.

Supported by National Institute of Child Health and Human Development Grant HD 07681.

Received for publication March 5, 1980.

Revised August 11, 1980.

Accepted August 28, 1980.

Reprint requests: Dr. Herbert E. Cohn, 2 Fenway Plaza, Boston, Massachusetts 02215.

**Present address: Program in Biology and Medicine, Brown University, Providence, Rhode Island.*

Table I. Maternal and fetal respiratory and hemodynamic data during hypoxemia (8% oxygen)

	Control	*Hypoxemia*	*Control*	*Hypoxemia (atropine pretreatment)*
Maternal arterial:				
Po_2 (torr)	89 ± 2	26 ± 1*	92 ± 4	28 ± 2*
Pco_2 (torr)	27 ± 2	29 ± 2	28 ± 2	33 ± 2
pH	7.52 ± 0.03	7.52 ± 0.03	7.50 ± 0.01	7.44 ± 0.03
Fetal carotid arterial:				
Po_2 (torr)	18 ± 2	9 ± 1	18 ± 1	7 ± 1†
Pco_2 (torr)	37 ± 1	38 ± 0	36 ± 1	42 ± 2
pH	7.44 ± 0	7.45 ± 0.03	7.43 ± 0.03	7.35 ± 0.04
Fetal femoral arterial:				
Po_2 (torr)	17 ± 1	7 ± 1*	16 ± 1	6 ± 1†
Pco_2 (torr)	38 ± 1	37 ± 4	42 ± 3	47 ± 3
pH	7.42 ± 0.01	7.42 ± 0.02	7.39 ± 0.02	7.33 ± 0.02†
Fetal heart rate (bpm)	189 ± 7	115 ± 5*	188 ± 12	214 ± 6
Fetal arterial pressure (mean) (mm Hg)	55 ± 1	68 ± 2*	53 ± 2	78 ± 5†

Gestational age: 127.2 ± 0.8 days; fetal weight: 2.18 ± 0.14 kg; n = 6.
$*p < 0.01$.
$\dagger p < 0.05$.

the fetal heart rate on quantitative changes in cardiac output and blood flows to fetal tissue during different levels of hypoxemia.

Methods

Animal preparation and methodology. We studied surgically prepared fetuses of 12 Dorset ewes at dated gestational ages of 124 to 130 days. Each ewe was fasted for 36 hours prior to surgery; anesthesia was induced with thiamylal sodium and maintained with halothane and oxygen by endotracheal tube. Following previously described principles for in utero fetal surgery,[8, 9] we inserted catheters in the fetal carotid artery, jugular vein, femoral artery and vein, and amniotic cavity by an aseptic technique. In eight of 12 fetuses, we placed a cuff type of electromagnetic flow transducer* on the fetal descending aorta through a retroperitoneal approach. The abdomen of the ewe was closed and a maternal femoral artery was cannulated. The catheters and the transducer cable were brought subcutaneously to the flank of the ewe and protected by a cloth pocket. The ewe and fetus recovered during the next 2 to 4 days postoperatively.

Fetal heart rate, arterial pressure, amniotic pressure, and descending aortic blood flow were recorded continuously with pressure transducers, a direct-writing recorder,† a cardiotachometer, and an electromagnetic flowmeter.‡

Po_2, Pco_2, and pH were measured with a blood gas analyzer* in simultaneously drawn maternal and fetal arterial blood samples. Fetal cardiac output and quantitative organ blood flows were measured in every fetus by the injection of radionuclide-labeled microspheres.[10] Spheres 15 μ in diameter with different labels were injected simultaneously into the superior and/or inferior vena cava. Reference samples were withdrawn continuously by syringe pumps from fetal carotid and femoral arteries during the microsphere injections. The rate of withdrawal was approximately 7 ml/min for 1.25 minutes. The withdrawal volume was measured precisely by determining syringe weight; the collection period was timed by stopwatch. Sufficient numbers of microspheres were collected in the reference samples and in all fetal tissues to permit accurate measurement of blood flow. The volume of blood withdrawn (3% to 4% of the estimated fetoplacental blood volume) was replaced with heparinized maternal blood. At the end of the experiment, the ewe and fetus were killed with an overdose of pentobarbital. The fetal organs and placenta were processed as previously described. The amounts of individual nuclide in each tissue were determined with a well-type gamma scintillation counter† and a 400-channel pulse height analyzer.‡ Calculation of organ blood flows, cardiac output and its distribution were performed on a computer. Statistical evaluations were made with each fetus serv-

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*C & C Instruments, H312 Venice Boulevard, Los Angeles, California 90066.

†Model 8800, Hewlett-Packard, 175 Wyman St., Waltham, Massachusetts 02154.

‡Mediflow ABC-1000, Omnicraft, Route 5, Box 5550, Auburn, California 95603.

*Corning Model 165, Corning Glass Works, Medfield, Massachusetts 02652.

†Nuclear-Chicago Corp., 2000 Nuclear Dr., Des Plaines, Illinois 60018.

‡Technical Measurement Corp., 441 Washington Ave., North Haven, Connecticut.

Table II. Fetal hemodynamic data during hypoxemia (8% oxygen)

	Control	*Atropine*	*Hypoxemia (atropine pretreatment)*
Cardiac output (ml/min/kg)	490 ± 29	454 ± 39	265 ± 23*
Stroke volume (ml/kg)	2.66 ± 0.14	2.20 ± 0.24	1.26 ± 0.08*
Umbilical blood flow (ml/min/kg)	207 ± 14	200 ± 20	165 ± 19†
Organ blood flows‡ (ml/min/100 gm tissue):			
Myocardial	131 ± 29	154 ± 36	385 ± 70†
Cerebral	98 ± 15	76 ± 15	124 ± 15
Adrenal	368 ± 79	393 ± 67	1224 ± 120*
Visceral	66 ± 5	56 ± 8	12 ± 7*
Splenic	376 ± 67	289 ± 65	25 ± 22†
Renal	163 ± 24	162 ± 14	32 ± 11*
Musculoskeletal	21 ± 2	20 ± 2	4 ± 1*
Pulmonary	64 ± 13	48 ± 19	11 ± 6

*$p < 0.01$.
†$p < 0.05$.
‡Myocardial and cerebral flows were estimated with femoral arterial blood used as a reference sample.

Table III. Fetal hemodynamic data during hypoxemia (8% oxygen)*

	Control	*Hypoxemia*
Heart rate (bpm)	158 ± 18	110 ± 16†
Arterial pressure (mm Hg)	58 ± 6	74 ± 2
Cardiac output (ml/min/kg)	416 ± 35	211 ± 49
Stroke volume (ml/kg)	2.65 ± 0.07	1.91 ± 0.06
Umbilical blood flow (ml/min/kg)	187 ± 43	114 ± 49
Organ blood flows‡ (ml/min/100 gm tissue):		
Myocardial	91 ± 41	359 ± 29
Cerebral	60 ± 5	85 ± 8
Adrenal	155 ± 62	337 ± 152
Visceral	75 ± 10	12 ± 6†
Splenic	387 ± 140	46 ± 35
Renal	143 ± 14	35 ± 3
Musculoskeletal	14 ± 1	4 ± 0†
Pulmonary	111 ± 33	24 ± 5

*Gestational age: 127 ± 2 days; fetal weight: 2.45 ± 0.18 kg; $n = 2$.
†$p < 0.05$.
‡Myocardial and cerebral flows were estimated with femoral arterial blood used as a reference sample.

ing as its own control by means of a paired t test.

Maternal hypoxemia was induced by the administration of a gas mixture containing oxygen (8% and 10% as specified under "Experimental design"), 3% carbon dioxide, and nitrogen to the standing ewe as described previously.

Parasympathetic blockade was induced in the fetus by injection of atropine; the efficacy of this blockade was assessed on the basis of inhibition of the heart rate response to acetylcholine. As atropine (approximately 0.2 mg/kg) was infused into the fetus, the fetal heart rate, arterial pressure, and descending aortic blood flow were recorded continuously. After the atropine blockade was established and these values had become steady, the baseline measurements were repeated. Since the atropine blockade lasted only 10 to 15 minutes, the fetuses were rechallenged with the parasympathetic agonist acetylcholine; if the unblocked response was observed, atropine was again administered.

Experimental design. The 12 fetuses, in two groups, underwent physiologic studies during different hypoxic states, both alone and in the presence of a parasympathetic blockade with atropine.

Group 1 (maternal inspiration of 8% oxygen, eight fetuses). We studied six fetuses in each of two experimental periods on separate days. During the first day, each fetus was observed at rest and during maternal hypoxemia. In the second period, 48 hours later, each fetus was studied at rest, during atropine blockade, and during hypoxemia in the presence of atropine blockade. Continuous flow transducer recordings of fetal descending aortic blood flow were made during both days of this study; microsphere measurements of fetal cardiac output were made during the second day only, at each of the three physiologic states described above.

We studied two additional fetuses with microsphere measurements of cardiac output at rest and during hypoxemia alone.

Group 2—Part A (maternal inspiration of 10% oxygen, four fetuses). We studied each fetus in individual experiments on each of 2 days, separated by a 48-hour interval. On the first day, each fetus was studied during a control period and during each of two experimental states: hypoxemia alone and hypoxemia in the presence of an atropine blockade. On the second day (48 hours later), the sequence of experimental manipulations was reversed, e.g., if hypoxemia alone had been followed by hypoxemia in the presence of an atropine blockade on the first day, then hypoxemia in the presence of an atropine blockade was followed by hypoxemia alone on the second day of study. This overall sequence of events was reversed in consecutive fetuses. Continuous flow transducer recordings of fetal descending aortic blood flow were made on both days of the study. Microsphere measurements of fetal cardiac output were made only during the second day of study, at each of the three physiologic states described above.

Group 2—Part B (maternal inspiration of 8% oxygen, four fetuses). This part of the study was conducted in the same animals used for Part A after completion of those experiments and allowance of time for recovery of the fetuses. We exposed each fetus to maternal hypoxemia (8% oxygen). Five minutes after the induction of maternal hypoxemia, each fetus received a bolus of atropine through the catheter in the inferior vena cava, while recordings of fetal heart rate and descending aortic blood flow were continued. Following termination of hypoxemia, each fetus was monitored through the recovery period.

Results

Group 1. A comparison of the fetal response to hypoxemia (maternal inspiration of 8% oxygen) on the first experimental day (phase 1) and the response to an identical hypoxemic stress in the presence of a fetal atropine blockade on day 2 (phase 2) is shown in Table I. Data are expressed as mean ± SEM. There were no significant differences in control values for maternal and fetal arterial blood gases and pH, fetal heart rate, and arterial pressure between the two phases of this study, and the control values were in the normal range for the gestational age studied.

During phase 1 (hypoxemia alone) maternal arterial Po_2 decreased to 26 torr (range 24 to 29) within 4 minutes after the ewe began breathing the low-oxygen mixture; this was associated with a fetal femoral arterial Po_2 of 7 torr (range 4 to 10). Fetal arterial Pco_2 and pH remained normal during hypoxemia. The fetal heart rate began to fall within 2 minutes after the ewe began breathing the 8% oxygen mixture and by 3 minutes after the induction of hypoxemia remained steady at a level 41% below control. Fetal arterial pressure increased by 24% ($p < 0.01$). These changes were paralleled by a 29% decrease in fetal descending aortic blood flow at 5 minutes ($p < 0.05$) (Fig. 1, *a*).

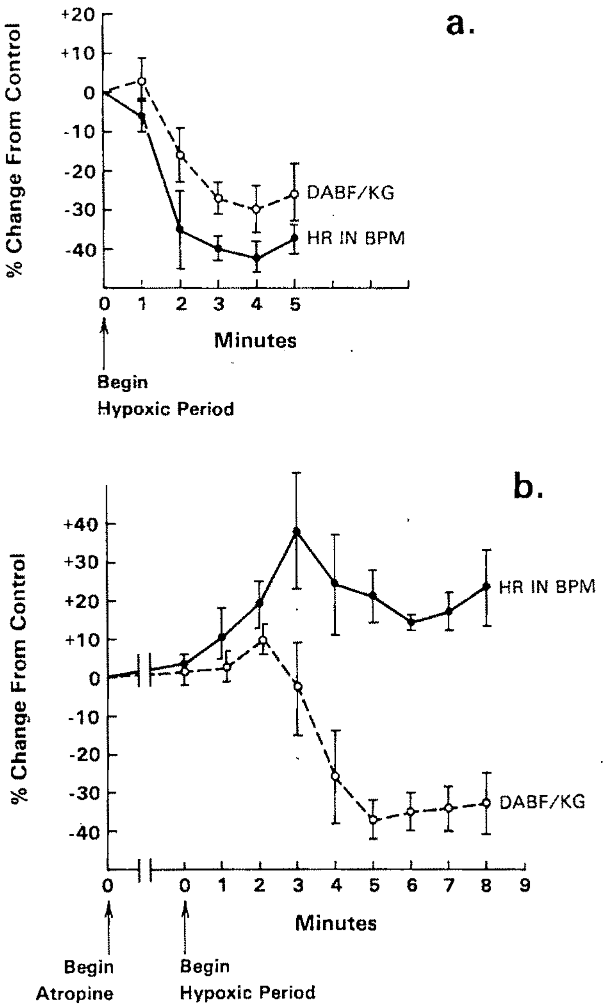

Fig. 1. Changes in fetal heart rate *(HR)* and descending aortic blood flow *(DABF/KG)* during hypoxemia (8% oxygen) *(a)* and during hypoxemia (8% oxygen) preceded by parasympathetic blockade with atropine *(b)*. Data are expressed as mean ± SEM (n = 4).

The administration of atropine to the fetus at rest, at a dose (approximately 0.2 mg/kg) which effectively blocked the chronotropic response to acetylcholine, led to a slight increase in fetal heart rate and descending aortic blood flow. As hypoxemia (8% oxygen) was induced in the presence of the fetal atropine blockade, maternal arterial Po_2 decreased to 28 torr (range 22 to 36); this was associated with a fetal femoral arterial Po_2 of 6 torr (range 3 to 10). Fetal pH and Pco_2 remained normal. During the first 3 minutes of hypoxemia, the fetal heart rate increased from 200 to 256, then returned to approximately 210 bpm. Mean fetal arterial pressure increased by 47% ($p < 0.05$). During 5 minutes of hypoxemia, the fetal descending aortic blood flow increased briefly, then dropped by 37% ($p < 0.01$) and remained low (Fig. 1, *b*).

In Table II hemodynamic and quantitative blood flow data for fetal tissues (based on microsphere injections) for the second phase of the study are shown. No

Table IV. Maternal and fetal respiratory and hemodynamic data during hypoxemia (10% oxygen)

	Control	*Hypoxemia*	*Control*	*Hypoxemia (atropine pretreatment)*
Maternal arterial:				
Po_2 (torr)	91 ± 2	33 ± 1*	89 ± 2	32 ± 1*
Pco_2 (torr)	30 ± 1	32 ± 1	29 ± 1	31 ± 1†
pH	7.49 ± 0.01	7.50 ± 0.01	7.50 ± 0	7.51 ± 0.01
Fetal femoral arterial:				
Po_2 (torr)	18 ± 1	9 ± 0*	17 ± 1	8 ± 1*
Pco_2 (torr)	45 ± 1	43 ± 1†	45 ± 2	44 ± 1
pH	7.42 ± 0.01	7.43 ± 0.01	7.42 ± 0.01	7.41 ± 0.01
Fetal heart rate (bpm)	172 ± 5	114 ± 5*	172 ± 7	208 ± 5
Fetal arterial pressure (mean) (mm Hg)	58 ± 1	64 ± 1*	59 ± 1	70 ± 3*

Gestational age: 127.5 ± 1.32 days; fetal weight: 2.51 ± 0.31 kg; n = 4.
*$p < 0.01$.
†$p < 0.05$.

Table V. Fetal hemodynamic data during hypoxemia (10% oxygen)

	Control	*Hypoxemia*	*Hypoxemia (atropine pretreatment)*
Cardiac output (ml/min/kg)	486 ± 42	468 ± 65	570 ± 61
Stroke volume (ml/kg)	2.74 ± 0.29	4.23 ± 0.53	2.76 ± 0.27
Umbilical blood flow	209 ± 13	223 ± 16	240 ± 8*
Organ blood flows† *(ml/min/100 gm tissue):*			
Myocardial	134 ± 45	356 ± 44‡	546 ± 115*
Cerebral	123 ± 35	169 ± 33	242 ± 20*
Adrenal	217 ± 19	893 ± 136*	733 ± 231
Visceral	71 ± 8	55 ± 22	92 ± 30
Splenic	320 ± 72	316 ± 152	177 ± 91
Renal	140 ± 8	138 ± 25	170 ± 47
Musculoskeletal	20 ± 3	15 ± 3	18 ± 4
Pulmonary	50 ± 19	10 ± 2	13 ± 2

*$p < 0.05$.
†Myocardial and cerebral flows were estimated with femoral arterial blood used as a reference sample.
‡$p < 0.01$.

significant change in fetal cardiovascular function or in the pattern of blood flow distribution occurred during atropine administration under normoxic conditions. Five to six minutes after the induction of hypoxemia, while the flowmeter indicated a 37% decrease in descending aortic blood flow, cardiac output measured by the microsphere technique had decreased by 46% ($p < 0.01$); stroke volume decreased by 53% ($p < 0.01$), and umbilical blood flow decreased by 20% ($p < 0.05$). Blood flow to the myocardial and adrenal tissues (ml/min/100 gm of tissue) increased significantly, while blood flow to the visceral, splenic, renal, and musculoskeletal tissues decreased significantly. The proportion of cardiac output distributed to the heart, brain, and adrenal tissues increased collectively from 6% to 18% (not significant) while the proportion distributed to the visceral, musculoskeletal, and pulmonary tissues decreased collectively from 52% to 18% ($p < 0.01$).

The hemodynamic data of two fetuses subjected to hypoxemia (8% oxygen *without* atropine pretreatment) and studied with microsphere injections are shown in Table III. There were no significant differences in fetal and maternal arterial Po_2, Pco_2, and pH between these fetuses and those described above during control periods and in response to maternal hypoxemia. During hypoxemia, fetal cardiac output decreased by 49% and stroke volume decreased by 28%. Umbilical blood flow decreased by 39%. The percentage of combined ventricular output distributed to the heart, brain, and adrenal tissues increased from 5% to 22% (not significant) while the amount distributed to the visceral, musculoskeletal, and pulmonary tissues decreased from 50% to 25% ($p < 0.01$).

Group 2—Part A. A comparison of the fetal response to hypoxemia (maternal inspiration of 10% oxygen) and the response to an identical hypoxemic stress in the presence of a fetal atropine blockade is shown in Table IV.

Table VI. Maternal and fetal respiratory data during hypoxemia (8% oxygen)

	Control	*Hypoxemia*
Maternal arterial:		
Po_2 (torr)	87 ± 2	24 ± 1*
Pco_2 (torr)	30 ± 1	33 ± 1
pH	7.48 ± 0.01	7.44 ± 0.01
Fetal femoral arterial:		
Po_2 (torr)	18 ± 2	4 ± 0*
Pco_2 (torr)	44 ± 1	44 ± 2
pH	7.38 ± 0.03	7.35 ± 0.03†

*$p < 0.01$.
†$p < 0.05$.

During the fetal response to hypoxemia alone, there was a decrease in maternal arterial Po_2 to 33 torr (range 29 to 38) within 4 minutes after the ewe began breathing 10% oxygen; this was associated with a fetal femoral arterial Po_2 of 9 torr (range 8 to 11). As the fetuses became hypoxemic, fetal heart rate decreased by 34% ($p < 0.01$) and the mean arterial pressure increased by 11% ($p < 0.01$). The fetal descending aortic blood flow decreased gradually by 23% over a 5-minute period (not significant) (Fig. 2, *a*).

The administration of atropine (0.2 mg/kg) to the fetus led to a slight increase in both fetal heart rate and descending aortic blood flow. During the fetal response to hypoxemia (10% oxygen) in the presence of the fetal atropine blockade, maternal arterial Po_2 decreased to 32 torr (range 29 to 36); this was associated with a fetal femoral arterial Po_2 of 8 torr (range 6 to 9). As the fetuses became hypoxemic, fetal heart rate remained very close to its prehypoxemic level. Arterial pressure increased by 18% ($p < 0.01$). Fetal ascending aortic blood flow increased very slightly over the first 3 minutes, then decreased very slightly (but not significantly) by 5 minutes of hypoxemia (Fig. 2, *b*).

Hemodynamic and quantitative blood flow data from the fetal response to hypoxemia (10% oxygen) alone and in the presence of a fetal atropine blockade are shown in Table V.

During the response to hypoxemia alone, fetal cardiac output decreased slightly but not significantly; stroke volume increased by 54% ($0.05 < p < 0.10$). Umbilical blood flow increased slightly but not significantly. Blood flow to the myocardial and adrenal tissues (ml/min/100 gm of tissue) increased significantly. The proportion of cardiac output distributed to the heart, brain, and adrenal tissues increased collectively from 8% to 15%, while the proportion distributed to the visceral, musculoskeletal, and pulmonary tissues decreased from 49% to 37%; neither set of changes was statistically significant.

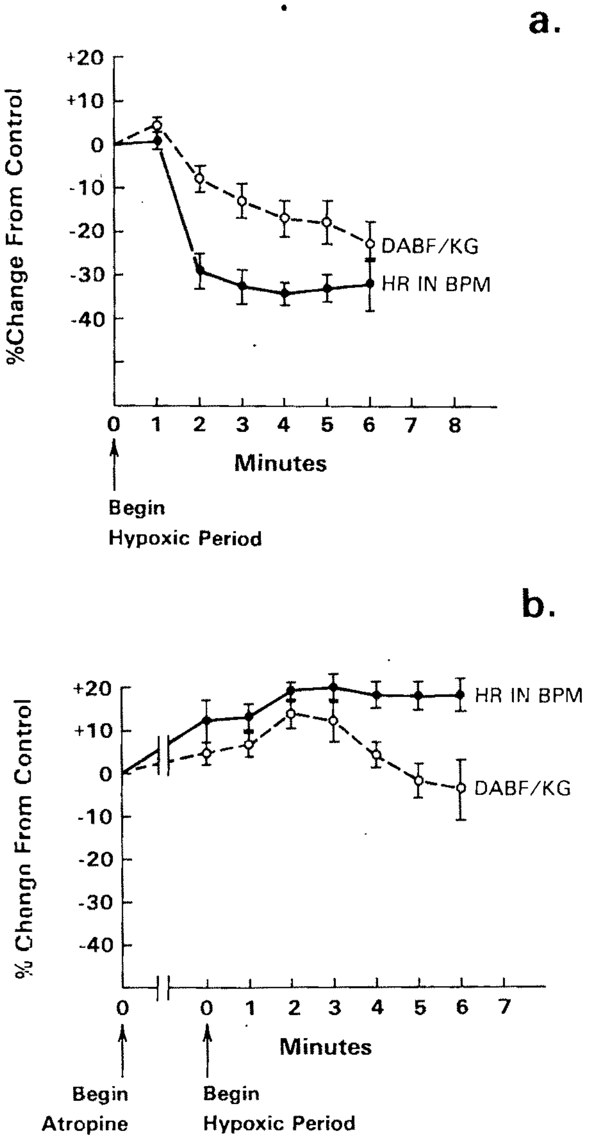

Fig. 2. Changes in fetal heart rate *(HR)* and descending aortic blood flow *(DABF/KG)* during hypoxemia (10% oxygen) *(a)* and during hypoxemia (10% oxygen) preceded by parasympathetic blockade with atropine *(b)*. Data are expressed as mean ± SEM (n = 4).

During the fetal response to hypoxemia (10% oxygen) in the presence of a fetal atropine blockade, there were no significant changes in fetal cardiac output or stroke volume. Umbilical blood flow increased by 15% ($p < 0.01$). Blood flow to the myocardial and cerebral tissues (ml/min/100 gm of tissue) increased significantly. The proportion of cardiac output distributed to the heart, brain, and adrenal tissues increased from 8% to 20% ($p < 0.01$) while the proportion distributed to the visceral, musculoskeletal, and pulmonary tissues decreased from 49% to 38% (not significant).

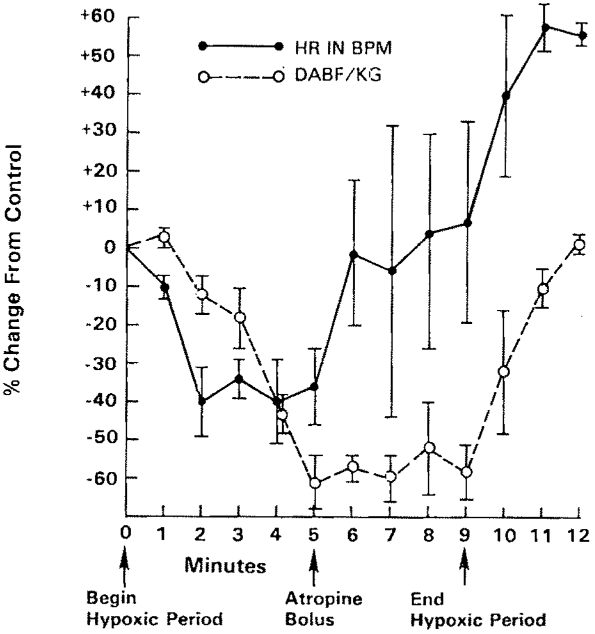

Fig. 3. Changes in fetal heart rate *(HR)* and descending aortic blood flow *(DABF/KG)* during hypoxemia (8% oxygen); an intravenous bolus of atropine was administered to each fetus 5 minutes after the induction of hypoxemia. Data are expressed as mean ± SEM (n = 4).

Group 2—Part B. The arterial blood gas data of fetuses that received an atropine bolus following the induction of hypoxemia (8% oxygen) are shown in Table VI. There was a decrease in maternal arterial Po_2 to 24 torr (range 22 to 26); this was associated with a fetal femoral arterial Po_2 of 4 torr (range 4 to 5). As the fetuses became hypoxemic, fetal heart rate decreased from 195 to 110 bpm and the descending aortic blood flow decreased by 58% ($p < 0.01$) (Fig. 3). When the atropine bolus was administered into the fetal inferior vena cava, the fetal heart rate increased abruptly to the prehypoxemic level; the descending aortic blood flow increased slightly but remained at approximately 50% below its control level until the hypoxemic period was ended. At that time, the fetal heart rate increased to 60% above its control value while the descending aortic blood flow returned to its control value.

Comment

In keeping with current evidence of significant physiologic alterations in acute fetal experimentation, we performed the present studies in chronic fetal preparations.[11-13] Control values for the respiratory and hemodynamic parameters which we studied were appropriate for gestational age, and there were no significant differences in the control values among groups of experiments.

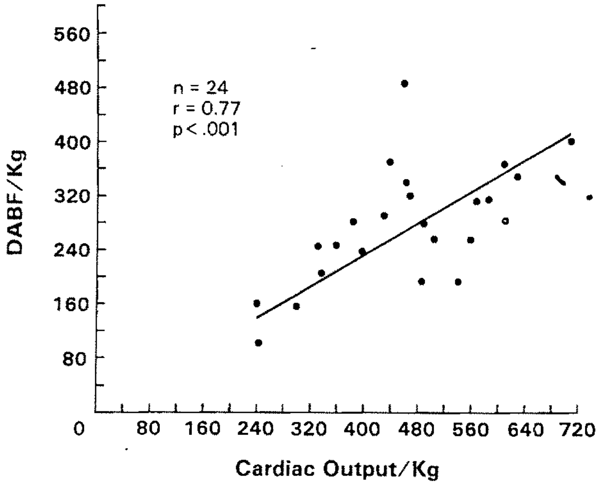

Fig. 4. Relationship of descending aortic blood flow *(DABF/Kg)* measured by flowmeter to *Cardiac Output/Kg* measured by the microsphere technique. (n = 24 observations in eight fetuses.)

During microsphere measurements of blood flow to fetal tissues, a sufficient quantity of sphere was infused into the catheter in the inferior vena cava so that adequate numbers of spheres reached all fetal organs and reference samples in conformance with the statistical needs of the microsphere technique (at least 400 and usually over 1,500 spheres reached each organ).[14]

Methodologic factors may have influenced the measurement of certain organ blood flows. The use of spheres infused into the inferior vena cava only, as opposed to the simultaneous use of differently labeled microspheres infused into the superior and inferior vena cava, may have led to a slight underestimate of pulmonary blood flow, since one cannot determine the contribution of return via the superior vena cava to pulmonary blood flow. Additionally, the ligation of one carotid artery for catheter placement in Group 1 fetuses could have altered cerebral blood flow in those preparations.

Calculations of fetal organ blood flows were based on reference withdrawal samples from the fetal femoral artery in all preparations. In the Group 1 studies, both carotid arterial and femoral arterial reference withdrawals were made in four of six preparations, while in Group 2 studies, only femoral arterial reference withdrawals were made. Upon comparison of the blood flow data calculated with both femoral and carotid arterial reference withdrawals with those obtained with femoral arterial withdrawals only, overall cardiac output was slightly greater in the former case, but between these two methods there were no significant differences in the changes of cardiac output and organ blood flows in response to perturbations of the physiologic state. Since Group 2 fetuses had femoral arterial reference withdrawals only, we used this reference sample as a common basis for all calculations.

We have found in our data a correlation between changes in cardiac output and changes in descending aortic blood flow. This correlation is somewhat stronger ($r = 0.92$, $p < 0.001$) when microsphere data are used for cardiac output (CO/kg) and descending aortic blood flow (DABF/kg) (the latter was calculated as the sum of umbilical blood flow per kilogram plus lower body blood flow per kilogram) but is also significant ($r = 0.77$, $p < 0.001$) when microsphere data for cardiac output (CO/kg) are correlated with flowmeter data for descending aortic blood flow (DABF/kg). This correlation, depicted graphically in Fig. 4, provides a basis for the use of the continuously recorded descending aortic blood flow as an indicator of changes in cardiac output.

Previous studies have shown that during hypoxemia, overall fetal cardiac output does not change significantly[3]; more recently, we[6] reported that cardiac output did decrease significantly during hypoxemia. The present study sheds light on this paradox by showing that changes in cardiac output depend upon the level of fetal hypoxemia achieved. This is supported by the studies of Peeters and associates,[4] who demonstrated that the pattern of blood flow redistribution is different at different levels of hypoxemia. Therefore, in any discussion of the fetal response to hypoxemia, particularly when different studies are compared, it is necessary that the degree of hypoxemia achieved in the fetus be clearly defined. For purposes of this discussion, we arbitrarily define ranges of fetal hypoxemia as follows: (1) "mild" (fetal arterial Po_2, 12 to 14 torr), "moderate" (fetal arterial Po_2, 8 to 11 torr), and "severe" (fetal arterial Po_2, 4 to 7 torr).

We compared four related experimental conditions: (1) moderate hypoxemia alone, (2) moderate hypoxemia in which the usual fetal heart rate response was prevented by parasympathetic blockade, (3) severe hypoxemia alone, and (4) severe hypoxemia, in which the fetal heart rate response was prevented by parasympathetic blockade.

The fetus exposed to moderate hypoxemia developed a significant bradycardia which was completely prevented by atropine pretreatment. With hypoxemia alone cardiac output was unaltered and was slightly, but not significantly, elevated by the same degree of hypoxemia in the presence of vagal blockade by atropine. Descending aortic blood flow (flowmeter data) decreased modestly with some small differences in the flow pattern with and without atropine blockade, but none of these changes was statistically significant. With this (moderate) level of hypoxemic stress then, there is little apparent effect on fetal cardiac output whether bradycardia is allowed to develop or is artificially blocked so that the normal heart rate is maintained.

Even though cardiac output is maintained in the face of moderate hypoxemia, delivery of oxygen to fetal tissues may still be impaired as a result of decreased oxygen content in circulating blood. As has been previously described,[3] the fetus responds in this situation by shunting blood away from "low-priority" tissues, such as skeletal muscle, gut, and kidneys, to the "high-priority" tissues, such as heart, brain, adrenals, and placenta. Such a redistribution of flow did occur in our experiments, as shown in Table V. There were some variations in the flow distribution to individual tissues in the presence of hypoxemia alone versus hypoxemia in the presence of an atropine blockade; when viewed in toto, however, with the exception of myocardial blood flow, which is discussed below, no clear-cut differences in the pattern of flow distribution in response to hypoxemia alone and hypoxemia with atropine blockade were seen. Therefore, as was the case for cardiac output, the presence of a parasympathetic blockade appears to have little effect on flow distribution in response to this specific level of hypoxemia.

The fetus exposed to severe hypoxemia developed bradycardia and a significant decrease in cardiac output, a response quite different from that of the fetuses stressed by moderate hypoxemia. This decrease in cardiac output was not dependent on heart rate since pretreatment with atropine prevented the bradycardia seen normally during this level of hypoxemia but failed to prevent the change in cardiac output. Observations of descending aortic blood flow, which, as noted above, is an indicator of changes in cardiac output, also support this conclusion. With hypoxemia alone, descending aortic blood flow was maintained for 1 minute and then rapidly fell, reaching a low point at 5 minutes. In the presence of an established atropine blockade, normal descending aortic blood flow was maintained for 3 minutes after initiation of hypoxemia, then fell and reached a level similar to that without atropine by 5 minutes and was maintained thereafter. As graphically illustrated in Fig. 3, the administration of a bolus of atropine to the severely hypoxemic fetus restored the heart rate to control levels but did not have a significant effect on cardiac output. Thus, it seems quite clear that the decreased cardiac output secondary to severe hypoxemia is entirely independent of the bradycardia which normally occurs.

A comparison of blood flow distribution data for severe hypoxemia (Tables II and III) with those for moderate hypoxemia (Table V) immediately indicates that the redistribution of blood flow in the face of the low oxygen levels and decreased cardiac output was much more intense with regard to the *decrease* in blood flow to the "low-priority" tissues. Conversely, there was no greater *increase* in flow to the "high-priority" tissue with severe as compared with moderate hypoxemia. Umbilical blood flow decreased significantly, whereas with moderate hypoxemia there had been a slight increase in this parameter. There was also no increase in the absolute flow value per unit weight to the brain and to the heart in the presence of severe as compared with moderate hypoxemia. The fact that there is no further increase in blood flow to the vital organs in the face of a lower oxygen content in the blood definitely indicates that the compensatory mechanisms are beginning to fail. This constitutes a physiologic basis for defining severe hypoxemia. Redistribution of fetal blood flow in response to severe hypoxemia, as seen during moderate hypoxemia, occurred regardless of an atropine blockade.

Our results are in agreement with those of Parer,[15] who found that during maternal hypoxemia (maternal arterial $Po_2 = 32.5$ torr) an intravenous bolus of atropine to the hypoxemic fetus caused an immediate increase in fetal heart rate but no change in umbilical blood flow. We would expect that during this level of hypoxemia, which corresponds to our "moderate" group, umbilical blood flow would be well maintained regardless of any change in heart rate during the hypoxemic period and that the administration of atropine would not significantly affect umbilical blood flow or overall cardiac output.

The conclusion of Green and associates[7] that atropine administration during hypoxemia results in an increase in both left and right cardiac output must be viewed in light of the very mild degree of hypoxemia (fetal $Po_2 = 13.4$ torr) to which their fetuses were subjected. The changes in cardiac output which they reported were also quite small.

Having dismissed bradycardia as the cause of the marked decrease in fetal cardiac output occasioned by "severe" hypoxemia, we will speculate briefly on other possible causes. The fundamental changes which result in this decrease might be associated with alterations in preload or afterload affecting the fetal heart and/or alterations in the delivery of oxygen to the fetal myocardium.

A decrease in preload through a reduction in venous return to the fetal heart could account for a decrease in stroke volume and overall cardiac output, although we have no data to support this. There is some suggestion in this study that an increase in afterload may have been a factor, in that similar increases in arterial pressure were seen during moderate and severe hypoxemia while greater decreases in tissue blood flow were seen during severe as compared with moderate hypoxemia. This suggests that net peripheral vascular resistance might have been higher during severe hypoxemia.

Oxygen supply to the myocardium is determined by myocardial blood flow and arterial oxygen content. Fisher and associates[16] have shown, in the fetal lamb, that myocardial extraction of oxygen and glucose is equal to values obtained from healthy adults, despite the lower arterial levels of oxygen and glucose in the fetus, because of the higher level of fetal myocardial blood flow. As the arterial oxygen decreases during hypoxemia, oxygen delivery also decreases and can be compensated for initially by coronary vasodilatation and increased flow. Since myocardial blood flow was not higher in the presence of severe compared to moderate hypoxemia and the oxygen content of circulating blood was lower in the former instance, delivery of oxygen to the myocardium was presumably less and may thus have accounted for decreased cardiac performance.

It is noteworthy that myocardial blood flow was greater during hypoxemia in the presence of atropine blockade than during hypoxemia alone in both moderate and severe hypoxemia, although the difference was significant ($p < 0.05$) only for the group with moderate hypoxemia. A similar finding was reported in the recent work by Sola and associates[17] who found that, in newborn lambs, atropine prevented a bradycardia associated with hemorrhage and caused a relative increase in myocardial blood flow. This may indicate that the myocardial oxygen requirement for a given level of cardiac work is greater at a faster than at a slower rate and that myocardial blood flow is greater in response to greater metabolic demand with the faster rate. This rationale has been given previously to explain similar phenomena in adult animals.[18]

In summary, we conclude that the level of hypoxemia which the fetus experiences is the major determinant of its cardiovascular response to such an episodic stress. These data indicate that the administration of atropine to the severely hypoxemic fetus does not restore its depressed cardiac output although atropine does abruptly increase its heart rate. During moderate hypoxemia, the fetal adaptations to a diminished oxygen delivery function well and the use of atropine does not offer additional benefits to the fetus. Indeed, if the utilization of oxygen by the fetal myocardium is less efficient at a higher heart rate, as suggested, the maintenance of a baseline fetal heart rate secondary to atropine blockade may impose an added burden upon the fetus during a prolonged period of hypoxemic stress. Finally, since bradycardia of similar magnitude occurs in both moderate and severe fetal hypoxemia, this parameter does not serve as an accurate gauge of the level of fetal hypoxemia.

We thank Ms. Joan Luthy and Mr. Paul MacGarvey for their skillful technical assistance.

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Ultrasound-facilitated intrauterine transfusions

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Ultrasound-facilitated intrauterine transfusion was performed on 35 fetuses. Eleven fetuses were hydropic and less than 26 weeks' gestation at the time of the first intrauterine transfusion (IUT). Only two (18%) neonates survived. Twelve fetuses were not hydropic and less than 26 weeks' gestation at time of IUT. Six (50%) neonates survived. Corrected neonatal survival rates for three hydropic and nine nonhydropic fetuses transfused after 26 weeks were 100% and 78%, respectively. The presence of ascites documented by ultrasound is an adequate indication for an IUT and permitted earlier detection of sicker fetuses; however, as a therapeutic aid, ultrasound neither diminished the fetal morbidity and mortality associated with the procedure nor completely eliminated the need for radiography to confirm proper intra-abdominal localization of the transfusion tubing. (AM. J. OBSTET. GYNECOL. 138:1200, 1980.)

IN 1963, Liley[1] performed the first successful intrauterine transfusion (IUT). Adopting and modifying his method, 365 fetuses have been transfused since 1964 at the Boston Hospital for Women (BHW). Since 1977, a phased-array sector scanning ultrasound system (Varian V-3000)[2] has been used to aid in the performance of IUTs. Proposed advantages of continuous ultrasound visualization during an IUT include diminished catheter placement uncertainty, reduced risk of fetal morbidity and mortality, continuous ultrasound contact well away from the needle puncture site, and avoidance of ionizing radiation. In this article we present the initial experience with ultrasound-aided IUTs and compare its advantages and disadvantages to x-ray–facilitated IUTs.

In 1956, Bevis[3] introduced the use of a spectral absorption curve of amniotic fluid obtained at amniocentesis as a reliable guide to predict the subsequent development of neonatal kernicterus. Liley[4] confirmed that the severity of hemolytic disease of the newborn could be predicted by considering both the size and trend of the deflection in the spectral absorption curve at 450 mμ of serially obtained amniotic fluid. A population of fetuses in whom the optical density (ΔOD) at 450 mμ predicted intrauterine death (IUD) was identified. Freda[5] noted that optical density deflections greater than 0.3 were associated with imminent fetal death. Fig. 1 demonstrates the ominous fetal outcome zone for gestations greater than 28 weeks. Above each author's respective "intervention" line, IUT or delivery is warranted. The BHW-Umansky line will be described in the "Methods" section.

Queenan[6] evaluated amniotic fluid from nonsensitized patients in the midtrimester, noting that the upper limit of normal ΔOD at 450 mμ rises from early midtrimester to a peak of 0.10 to 0.11 at 23 to 26 gestational weeks. Liggins[7] recommended that at maturities less than 28 weeks, a ΔOD at 450 mμ between 0.3 and 0.35 would indicate repeat amniocentesis in 1 week rather than an IUT. In the midtrimester, Whitfield[8] considers IUT appropriate when the ΔOD at 450 mμ is

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Received for publication May 29, 1980.

Revised August 19, 1980.

Accepted August 28, 1980.

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above or moving in the direction of 0.3. Fig. 2 represents the ΔOD at 450 mμ in the midtrimester of unaffected pregnancies (according to Queenan) and the midtrimester transfusion lines established by the above authors.

Six years after Liley's pioneering procedure, Queenan[9] combined data from 15 medical centers on 1,074 x-ray–aided IUTs and noted that 81% of fetuses initially transfused prior to 26 weeks and 48% of fetuses initially transfused after 26 weeks died in utero. Subsequent reports of large series of x-ray–facilitated IUTs from single institutions[10-13] were confirmatory; however, Bowman,[14] advocating the use of IUT, reported that, whereas transfusion of a hydropic fetus prior to 26 weeks carried a 66% perinatal mortality rate, transfusion of a hydropic fetus after 26 weeks was associated with a perinatal mortality rate of only 49%. Additionally only 27 of 81 (33%) nonhydropic fetuses transfused prior to 26 weeks died.

Methods and material

From January 1, 1977, to December 30, 1979, 33 women (35 pregnancies) experienced 87 IUTs. The patients were divided into four groups. Group A patients were transfused prior to or at 26 weeks as a result of ultrasound-detected ascites. Group B patients were transfused prior to or at 26 weeks as a result of an elevated amniotic fluid ΔOD at 450 mμ but did not have fetal ascites. Group C patients were transfused after 26 weeks as a result of ultrasound-detected fetal ascites, and Group D patients were transfused after 26 weeks as a result of an elevated ΔOD 450 mμ and did not have fetal ascites.

Ultrasound evaluation was performed at 22 weeks or, if it occurred after 22 weeks, at the time of the initial referral. In these sensitized fetuses, ultrasound-detected ascites was an immediate indication for IUT.

If ascites was not noted, ultrasound-directed amniocentesis was performed if indicated by Coombs titer,[10, 15] and a ΔOD at 450 mμ was obtained, by means of scanning spectrophotometric analysis.[15] The frequency of repeat ultrasound and amniocentesis was based on previous reproductive history, antibody titer, or initial level of ΔOD. Usually repeat evaluations were carried out within 3 weeks; however, weekly tests often were necessary. Prior to 28 weeks a decision to transfuse was based on the relationship of one or more measurements of ΔOD at 450 mμ to a transfusion line constructed by one of the authors (I. U.)[15] (dotted line in Fig. 2). Specific values for this line include 0.19 at 21 weeks, rising to 0.36 at 26 weeks, and then declining to 0.30 at 28 weeks. After 28 weeks an IUT was indicated when the ΔOD at 450 mμ entered or was very close to entering zone III of Liley,[4] as modified at the Ross Conference on intrauterine transfusion in 1966, by raising the height of the intervention-transfusion line by 10%[15] (dotted BHW-Umansky line in Fig. 1). Specific values at 450 mμ were 0.30 at 28 weeks' gestation, 0.25 at 30 weeks' gestation, and 0.20 at 32 weeks' gestation.

The technique of ultrasound-aided IUT has been described in detail.[2] To summarize, the phased-array sector scanner (Varian V-3000) system is used to survey the maternal abdomen to determine the fetal lie, placental position, and optimal site and depth for proper needle placement. After a scalpel blade has nicked the locally anesthetized skin, a 15-gauge Tuohy needle is passed through the cleansed abdominal wall and uterine musculature. The Tuohy needle, under continuous observation of the ultrasound probe, which is moved perpendicularly on the maternal abdomen, is thrust into the fetal peritoneal cavity below the level of the

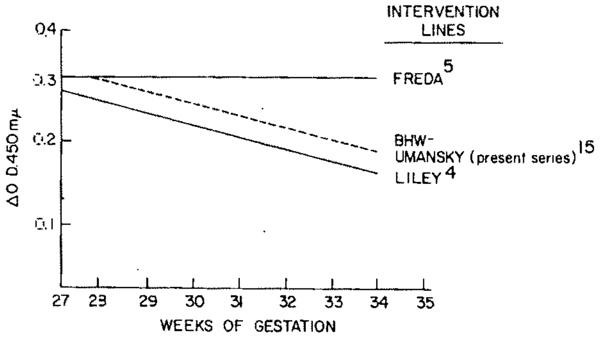

Fig. 1. ΔOD at 450 mμ above each author's intervention line would indicate IUT or delivery.

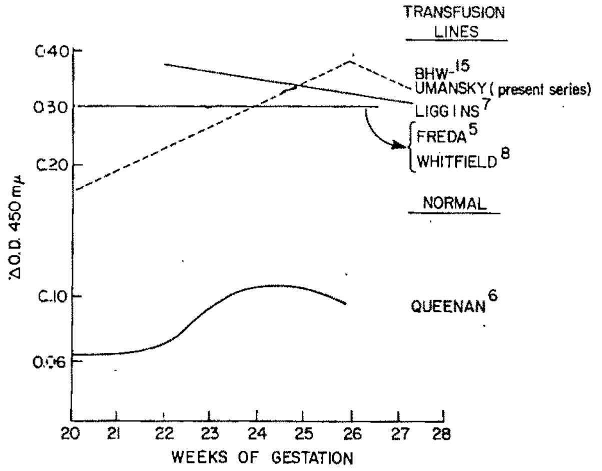

Fig. 2. ΔOD at 450 mμ in the midtrimester.

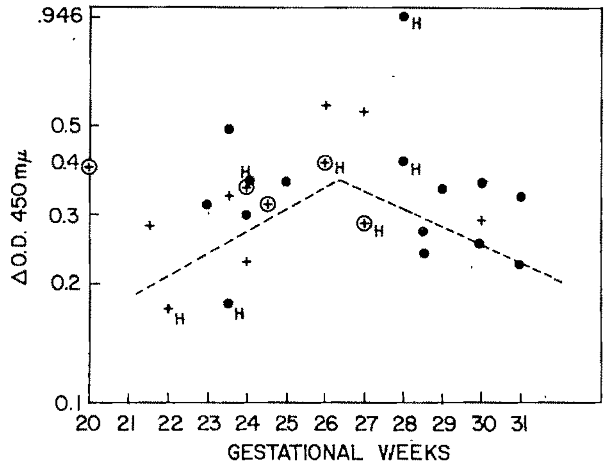

Fig. 3. Gestational week during which the first IUT was performed. *H* = Hydrops at the first IUT; the BHW-Umansky transfusion line is indicated by ----. Perinatal outcome: ● = Survived; ⊕ = intrauterine death; + = neonatal death.

fetal liver, spleen, and umbilicus. Five milliliters of saline is injected through the needle, serving as an ultrasound contrast agent and as preliminary confirmation of proper placement of the needle. Gentle aspiration serves to detect and remove all fetal ascites. Approximately 40 to 45 cm of epidural catheter,* with stylet removed, is threaded through the Tuohy needle, which is then withdrawn. Proper intraperitoneal localization is confirmed by injecting 5 ml of Hypaque and obtaining one coned-down x-ray film (estimated maternal skin dose = 400 millirads). The patient is then brought to the antepartum ward for the transfusion. To assure optimal red blood cell survival, diminish the chance of graft-versus-host disease,[16] and not overdistend the fetal peritoneal cavity, fresh, irradiated (5,000 rads†), 0-negative blood compatible with the mother's serum is used, in a volume calculated from the formula: Volume to be transfused = 100 ml − X, where X = 10 × (30 weeks − current gestational weeks). The fetal heart rate is continuously monitored during the IUT.

The patient may leave the hospital soon after completion of the transfusion. Prior to discharge, a repeat ultrasound examination confirms the localization of the blood within the fetal abdominal cavity and the presence of a living fetus.

For both hydropic and nonhydropic fetuses, the schedule for repeat IUTs requires individualization; however, most second IUTs are scheduled 7 days following the first; third IUTs follow the second by 7 to 10 days, and a fourth IUT, necessary in eight patients, occurred 14 to 21 days after the third.

Results

Of the 33 women, 14/32 (44%) had been sensitized to D in association with a delivery, abortion, or ectopic pregnancy in the United States after 1970, and 7/14 (50%) had not been given Rho (D) immune globulin.

The gestational week during which the first IUT was performed for all pateints who had analysis of the ΔOD at 450 mμ amniotic fluid is displayed in Fig. 3. The dotted line is the BHW-Umansky transfusion line described above. The three nonhydropic values below the "transfusion line" all represented individuals with either rapidly rising ΔODs and/or a previous stillbirth in the midtrimester.

The results of the IUTs are summarized in Table I.

Group A. Eleven fetuses were transfused 29 times. At initial IUT, ascites was confirmed in all fetuses. Five fetuses died in utero (two within 24 hours of the transfusion). Two nonfatal extraperitoneal traumatic Tuohy needle insertions were documented. Six live-born infants were delivered; however, only two babies survived (18% of all fetuses transfused in the group).

Group B. Twelve fetuses received 35 transfusions. Four fetuses died in utero, all within 24 hours of the IUT (three of four suffered documented trauma at the time of IUT). Five nonfatal, extraperitoneal traumatic Tuohy needle insertions were documented. Eight babies were born alive and six survived (50% of all transfused in the group).

Group C. Three fetuses were transfused seven times. Ascites was confirmed in all at the time of IUT. No fetus died in utero and two of three survived; however, the one baby who did not survive had congenital absence of the kidneys (100% corrected survival of all fetuses transfused in this group).

Group D. Nine fetuses received 16 IUTs. Two fetuses died in utero (one within 24 hours). One nonfatal, extraperitoneal traumatic Tuohy needle insertion occurred. Seven fetuses were born alive and all survived (78% of all transfused in this group).

In sum, 35 fetuses received 87 IUTs. Eleven of 35 fetuses (31%) died in utero, and seven died within 24 hours of the procedure.

All IUDs occurring within 24 hours of the IUT have been arbitrarily designated "IUT related." The total IUT-related deaths per number of IUTs was 7/78 or 8%, and the total IUT-related deaths per number of fetuses transfused was 7/35 or 20%. Eight unsuccessful and traumatic insertions of the Tuohy needle into the

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*Lumbar caudal epidural catheter, W stylet, 19 gauge, 36 inch, Deseret Pharm. Co., Sandy, Utah.

†Model M, Gammator, Isomedics, Inc., Parsippany, New Jersey.

Table I. Results of intrauterine transfusions at BHW, January, 1977 to December, 1979: Fetal condition and gestational age at initial transfusion

	≤26 Weeks—ascites (A)	*≤26 Weeks—no ascites (B)*	*>26 Weeks—ascites (C)*	*>26 Weeks—no ascites (D)*	*Total*
No. of fetuses	11	12	3	9	35
No. of IUTs	29	35	7	16	87
No. IUDs	5	4	0	2	11 (31%)
No. of IUDs within 24 hr. of procedure	2	4	0	1	7
No. of nonfatal traumatic Tuohy insertions	2	5	0	1	8
No. of live births	6	8	3	7	24
Corrected survival (% transfused fetuses)	2 (18%)	6 (50%)	2 (100%)	7 (78%)	17 (49%)

pleural space (4), retroperitoneal space (1), spinal canal (1), gastrointestinal tract (1), and "high-pressure bloody area" (1) were documented.

Comment

Fluoroscopy, as an aid in performing IUT,[1, 10–14] often required pretransfusion amniocentesis to inject a radiopaque solution and pretransfusion sedatives, analgesics, and cough suppressants. Fetal and maternal radiation exposure, although diminished by the image intensification technique, ranged from 0.24[16] to 10[12] rads and 4 to 30 rads,[13] respectively. The depth of fetal penetration could only be estimated. It was hoped that the introduction of ultrasound as a technique to replace fluoroscopy would permit accurate and early detection, treatment, and follow-up of severely affected fetuses, elimination of radiation exposure, and the diminishing of the morbidity and mortality associated with IUT.

As a diagnostic tool, ultrasound was accurate. Ultrasound-detected fetal ascites was always confirmed at IUT; therefore, if it was detected prior to 30 to 31 weeks, IUT was performed. Although fetal ascites is usually associated with a high ΔOD 450 mμ, this is not always true, and in the presence of polyhydramnios both the trend and absolute ΔOD might suggest procrastination. Reliance on ultrasound avoids this inappropriate delay.

For post-IUT follow-up, weekly ultrasound examination of all transfused fetuses permits observation of the rate of absorption of transfused blood from the fetal abdominal cavity, the onset or diminution of fetal ascites, the evaluation of polyhydramnios, and fetal movements.

It was also hoped that the use of ultrasound during IUT would significantly diminish the fetal morbidity and mortality associated with IUT. Of recent reports of x-ray–facilitated IUTs, only Bowman[14] includes data reported in a manner similar to our data. The results of the two experiences are statistically similar. A binomial test of proportions with the use of Bowman's[14] data for P and BHW data for numbers of successes and number of events yielded no significant differences.

Statistical evaluation of these risks among the four groups is not possible or appropriate; however, the risk to an individual fetus per IUT for each fetus can be calculated. An analysis of variance showed no statistically significant differences in either nonfatal insertions per number of IUTs ($F = 0.76$, $p = 0.52$) or IUT-related deaths per number of IUTs ($F = 1.90$, $p = 0.15$) for fetuses within any group; however, the small number of cases may have reduced the statistical power of the F test, so that significant differences may not be detectable.

It would appear that ultrasound-facilitated IUT decreased radiation exposure and procedure times and permitted earlier detection of sicker fetuses, which allowed salvage of individual fetuses possibly destined to have been midtrimester intra-uterine deaths; however, the overall perinatal mortality, which probably is as much related to the severity of fetal illness and prematurity as it is to the skill of the perinatal team, has not been shown by this report to be reduced by the introduction of this new technique.

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Interrelationships of human chorionic gonadotropin, human placental lactogen, and pregnancy-specific β_1-glycoprotein throughout normal human gestation

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Human chorionic gonadotropin (hCG), human placental lactogen (hPL), and pregnancy-specific β_1-glycoprotein (PSBG) were measured by radioimmunoassay in 270 samples of serum from women with uncomplicated pregnancies. All three proteins were significantly correlated with each other in individual samples of serum and with the estimated trophoblastic mass during the first trimester. No significant correlation could be demonstrated between the concentrations of hCG and PSBG in maternal serum during the second or third trimesters or between the concentrations of hCG and hPL during the second trimester. Levels of PSBG and hPL in serum were significantly correlated throughout all three trimesters. These findings suggest that the secretion of hCG, hPL, and PSBG may be regulated by similar control mechanisms during the first trimester of pregnancy. However, after this period, the factors that modulate the production of hCG differ from those that regulate the production of hPL and PSBG. (AM. J. OBSTET. GYNECOL. 138:1205, 1980.)

WHEN ONE CONSIDERS the totipotential nature of the trophoblast, one is not surprised that this tissue has been found to secrete a variety of different substances, including several proteins that appear to be pregnancy-specific.[1] The qualitative and quantitative patterns of secretion of two of these proteins, human chorionic gonadotropin (hCG) and human placental lactogen (hPL), have been well described.[2-5] Interest has also centered around a recently discovered trophoblastic protein, pregnancy-specific β_1-glycoprotein (PSBG, also SP_1).[6, 7] PSBG is a product of the syncytiotrophoblast and, like hCG, appears in the maternal bloodstream shortly after implantation.[8-10] The qualitative pattern of the concentrations of PSBG in maternal serum throughout pregnancy resembles that of hPL, with increasing concentrations being found as pregnancy progresses.[6, 7, 11-14] This is in contrast to the pattern found with hCG, which peaks between the eighth and tenth weeks of gestation, followed by a decrease to levels that are approximately one fifth to one tenth of the peak concentration.[2] These lower levels are maintained throughout the remainder of pregnancy.[2]

Since all three proteins are synthesized and secreted by the placental syncytiotrophoblast,[15-18] the discordant secretory patterns of hCG and the other two proteins suggest that the production of hCG is regulated differently than the synthesis and secretion of hPL and PSBG. Alternatively, the secretion of these proteins may be differentially modulated as pregnancy progresses. This study was designed to examine the interrelationships of these trophoblastic proteins throughout normal pregnancy.

Material and methods

Blood samples. Single samples of blood were obtained by venipuncture from 270 women who were participating in a study of natural family planning, or who attended the Obstetrical Clinic at Cedars-Sinai Medical Center. All pregnancies were judged to be normal at the time that the samples of blood were obtained. Dating of the pregnancy was based upon the

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Received for publication May 23, 1980.

Accepted August 13, 1980.

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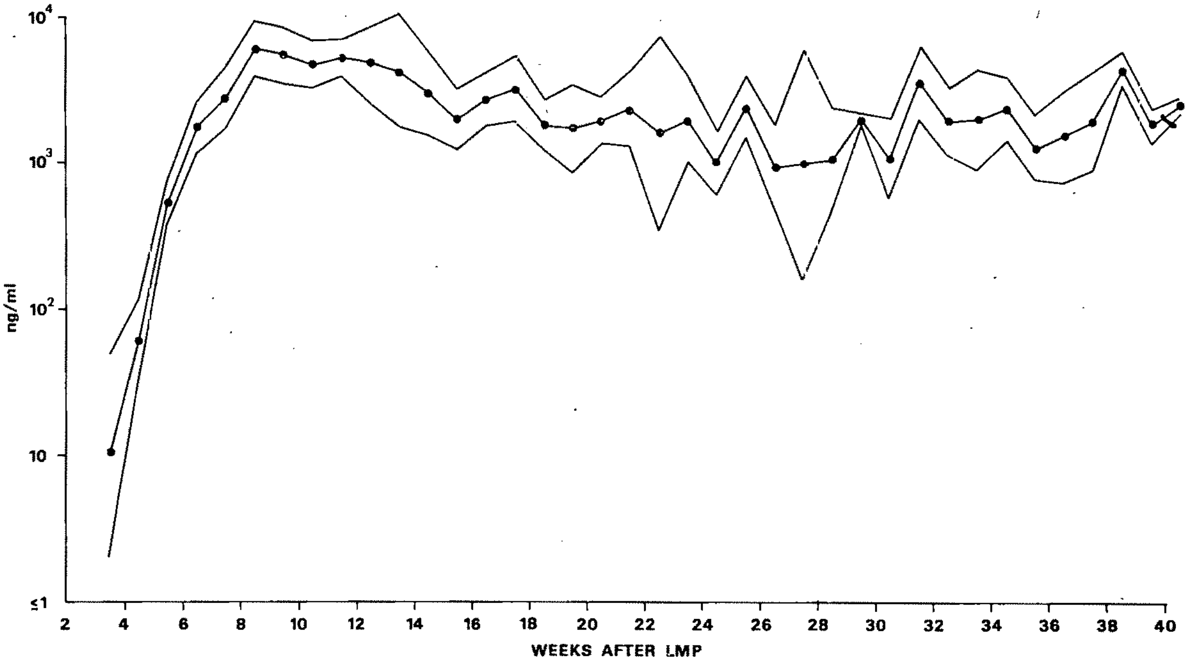

Fig. 1. Weekly concentrations of hCG in maternal serum throughout pregnancy. The solid dots represent the geometric mean, and the outer lines represent the 95% confidence limits. *LMP,* Last menstrual period.

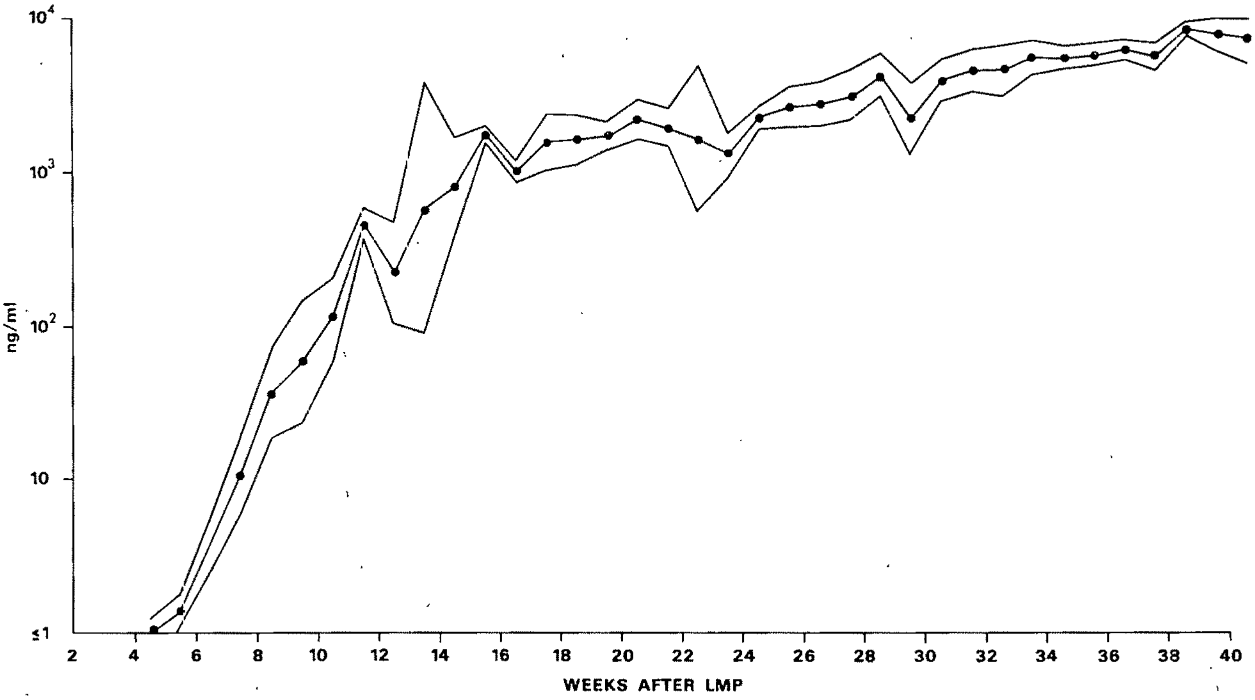

Fig. 2. Weekly concentrations of hPL in maternal serum throughout pregnancy. The solid dots represent the geometric mean, and the outer lines represent the 95% confidence limits. *LMP,* Last menstrual period.

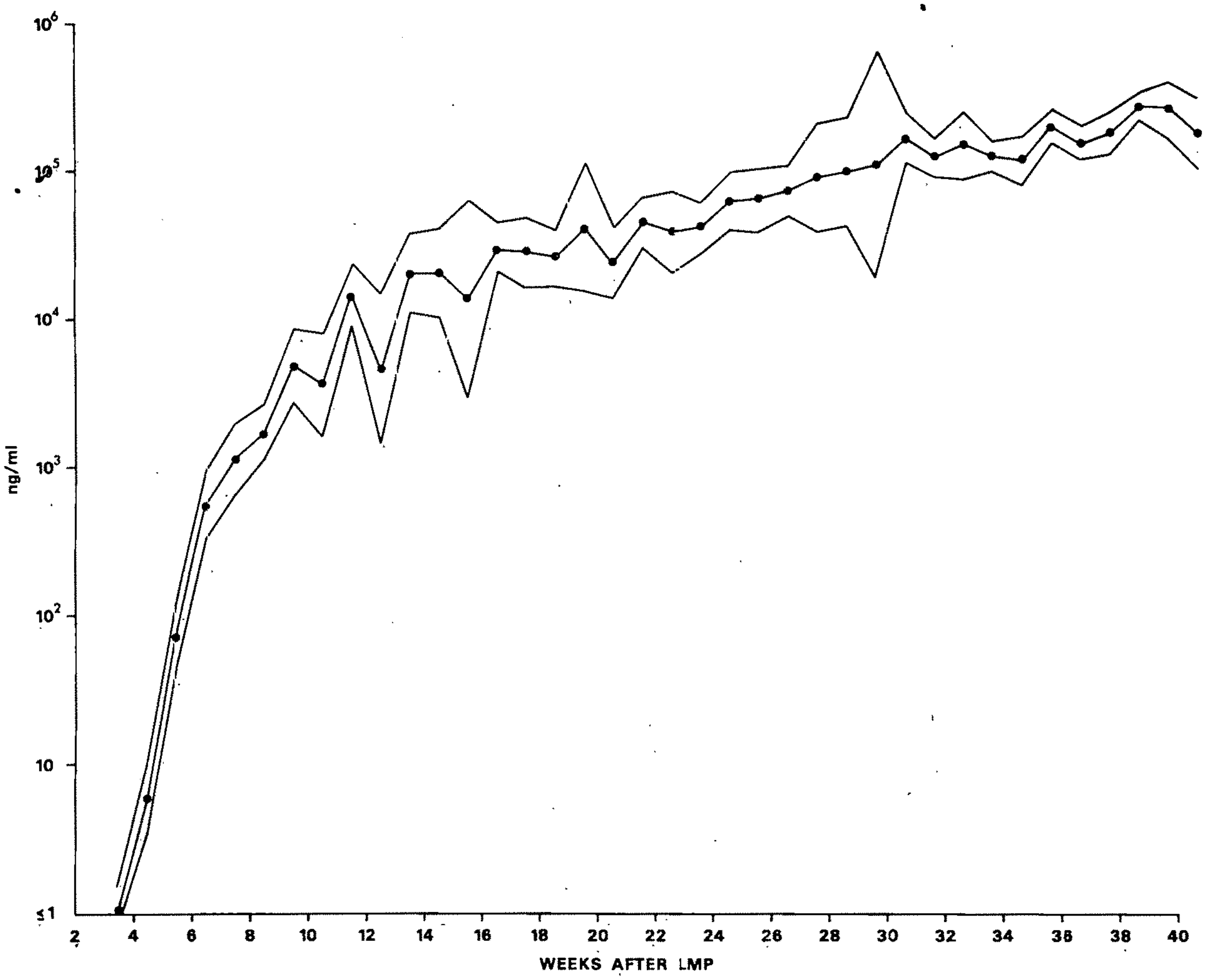

Fig. 3. Weekly concentrations of PSBG in maternal serum throughout pregnancy. The solid dots represent the geometric mean, and the outer lines represent the 95% confidence limits. *LMP*, Last menstrual period.

patient's last normal menstrual period. After the blood had been collected, it was centrifuged and the serum was separated and frozen at −20° C until assayed.

Radioimmunoassays. Previously described radioimmunoassay methods[19, 20] were used to measure hCG and PSBG. Measurement of hPL was made by double antibody radioimmunoassay with reagents obtained from the Hormone Distribution Officer, of the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland. All three assays have a minimum sensitivity of 1 to 2 ng/ml of serum. The intra-assay coefficients of variation for the hCG, PSBG, and hPL radioimmunoassays during the period of the study were 7.7%, 10%, and 11%, respectively, whereas the interassay coefficients of variation for each protein were 15.1%, 12.1%, and 13.9%, respectively. The statistical methods of Rodbard[21] were used for quality control and dose interpolation.

Estimation of trophoblastic mass. Estimates of mean trophoblastic mass were based upon determinations of placental weight performed by a number of investigators.[22–28] The percentage of placental mass occupied by the trophoblast was derived from the morphometric data of Vokaer and Eynde,[29] Martin,[30] and Laga and associates.[31]

Statistical analysis. The concentration of each of the placental proteins in serum conformed to a log-normal distribution, and, therefore, logarithmic transformation was carried out prior to statistical analysis. Spearman's rank order correlation coefficients and factor (principal components) analysis were carried out through the use of programs BMD P3S and BMD P4M at the Health Sciences Computing Facility, University

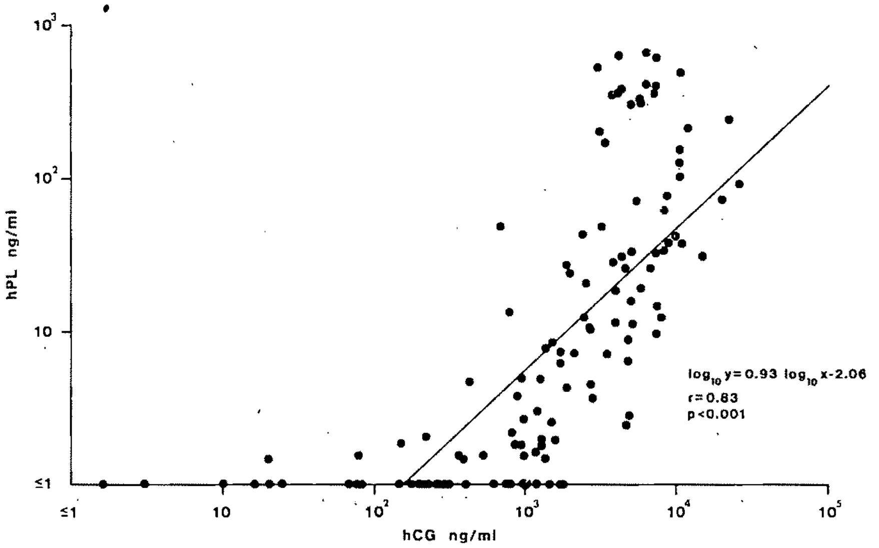

Fig. 4. Scatter plots and regression analysis of first-trimester concentrations of hCG and hPL in serum, measured in the same samples of serum.

Table I. Spearman's rank order correlation coefficients for hCG, hPL, and PSBG during each trimester

	Trimester					
	First		*Second*		*Third*	
Protein	*PSBG*	*hPL*	*PSBG*	*hPL*	*PSBG*	*hPL*
hCG	0.88*	0.83*	−0.29	−0.13	0.09	0.45†
hPL	0.87*	—	0.36†	—	0.38†	—

*$p < 0.001$.
†$p < 0.05$.

of California at Los Angeles, California. All levels of significance were corrected for multiple comparisons.

Results

The mean concentrations of hCG, hPL, and PSBG in maternal serum for each week of gestation are depicted in Figs. 1 through 3. A logarithmic increase in the concentration of each protein in serum was noticed during the first trimester of pregnancy. Peak levels of hCG were reached between 8 and 13 weeks after the last menstrual period, followed by a decline to relatively constant concentrations throughout the remainder of pregnancy (Fig. 1). Both PSBG and hPL demonstrated a continuous increase throughout pregnancy, with peak concentrations in serum being reached late in the last trimester (Figs. 2 and 3).

In order to explore the correlations between each of the placental proteins in the individual samples of serum, the data were grouped into trimesters and analyzed by Spearman's rank correlations. Division of the data into trimesters was performed in order to provide a sample size that was sufficient for statistical analysis. In addition, the correlation patterns were constant from month to month within each trimester. Therefore, analysis by trimesters reasonably reflected the relationship for the entire 3-month period.

The correlation coefficients between each of the proteins are given in Table I. All three proteins were significantly correlated with each other during the first trimester. Principal component analysis of the concentration of all three proteins in individual samples of serum indicated that 87% of the variance of the data was accounted for by a single linear combination in three-dimensional space. There was no significant correlation between concentrations of hCG and PSBG in maternal serum during the second and third trimesters, nor was there a correlation between concentrations of hCG and hPL during the second trimester. However, during the third trimester, hCG and hPL were significantly correlated. Both PSBG and hPL were significantly correlated throughout all three trimesters. The scatter plots corresponding to the correlations between the proteins during the first trimester are illustrated in Figs. 4 through 6.

The estimated trophoblastic mass of the placenta is illustrated in Fig. 7. Table II presents the correlation between estimated trophoblastic mass and concentrations of each placental protein in the maternal serum during each of the trimesters. All three proteins were

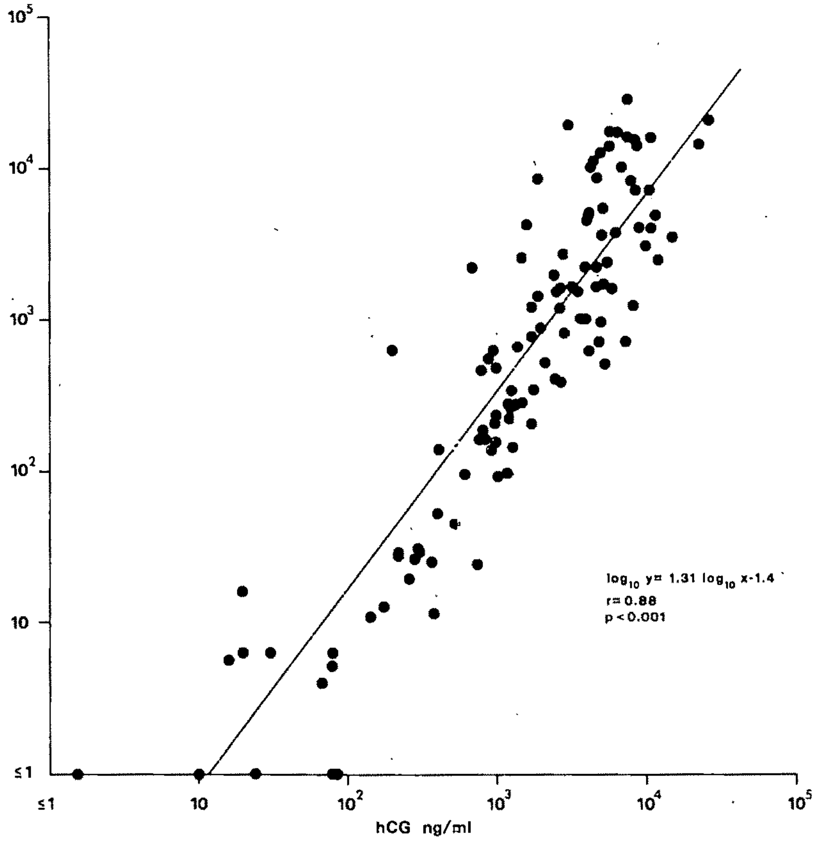

Fig. 5. Scatter plots and regression analysis of first-trimester concentrations of hCG and PSBG in serum, measured in the same samples of serum.

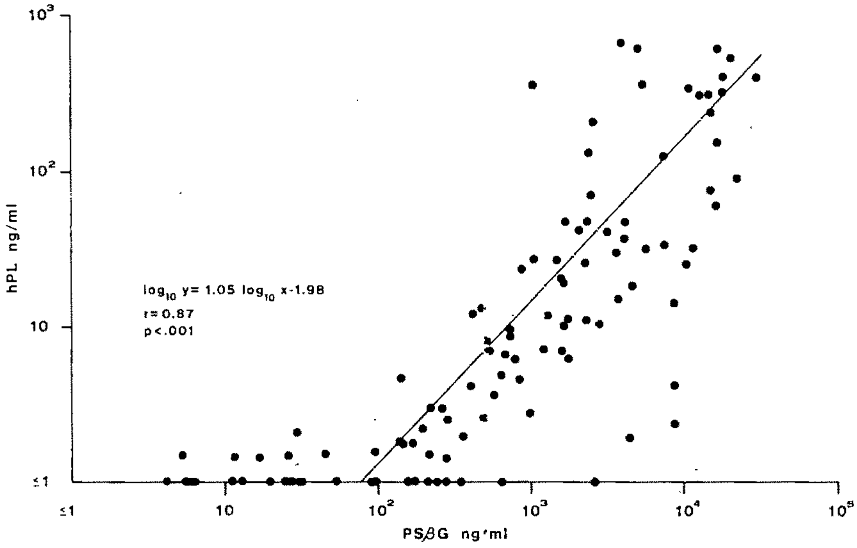

Fig. 6. Scatter plots and regression analysis of first-trimester concentrations of PSBG and hPL in serum, measured in the same samples of serum.

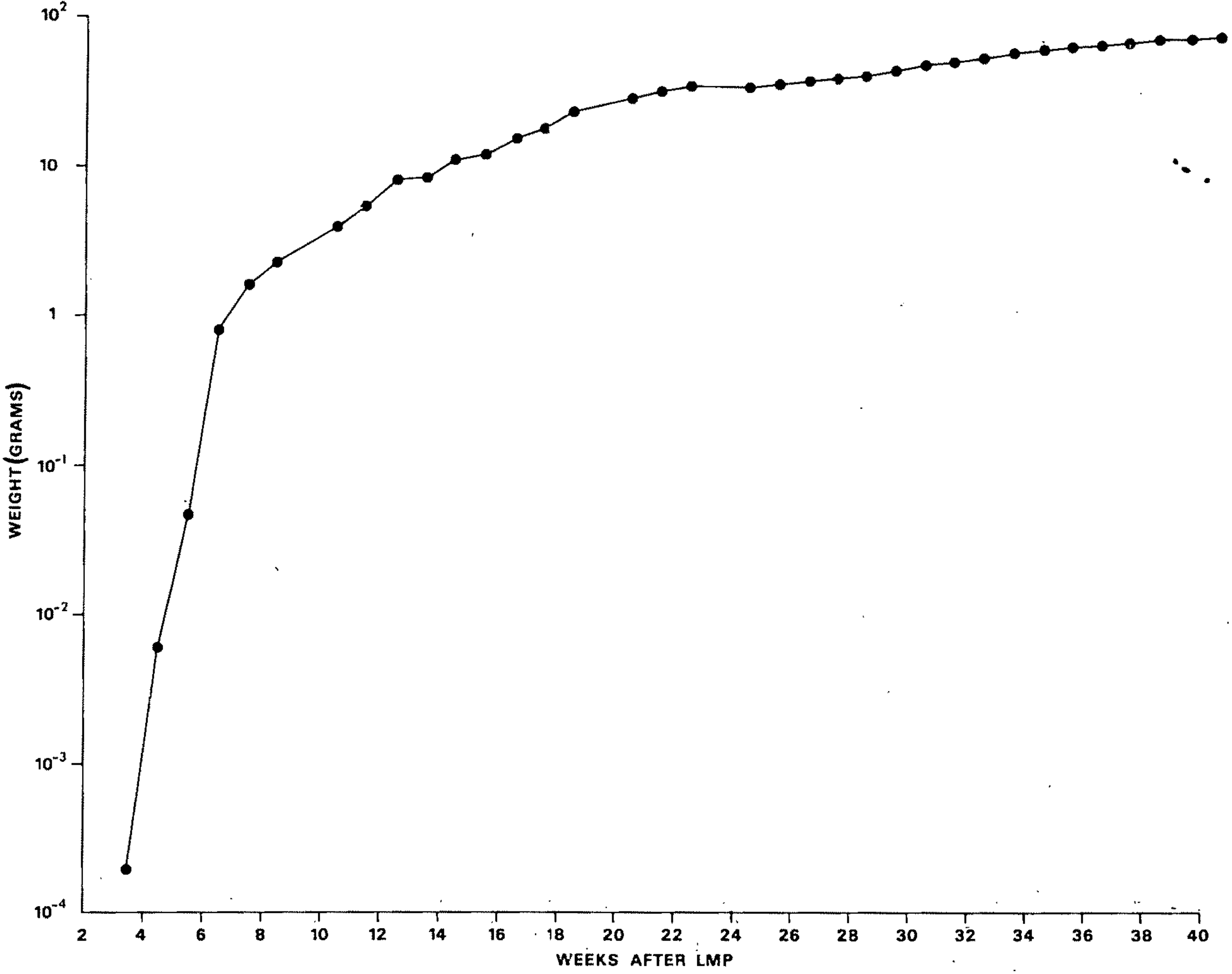

Fig. 7. Estimated trophoblastic mass, in grams, throughout normal pregnancy. Data derived from References 22 to 31. *LMP,* Last menstrual period.

significantly correlated with the estimated trophoblastic mass during the first trimester. This significant correlation was maintained for PSBG and hPL during the second and third trimesters of pregnancy. However, the correlation between trophoblastic mass and concentrations of hCG in maternal serum was not significant during the second and third trimesters of pregnancy.

In order to evaluate the usefulness of measurments of each of these trophoblastic proteins in maternal serum for the diagnosis of early pregnancy, the frequency of detection, by present methods, of each immunoreactive protein in women during early pregnancy was ascertained. As is illustrated in Fig. 8, hCG was detected in the serum of all pregnant patients 4 or more weeks after their last menstrual period. Five or more weeks were required for 100% positivity for PSBG, whereas hPL was not consistently detected in pregnant women until 8 or more weeks after the last menstrual period. Thus, with the assays used in the present study, measurements of hCG were more reliable than determinations of PSBG or hPL for the early diagnosis of pregnancy.

Comment

The strong correlation between the concentrations of hCG, hPL, and PSBG in individual samples of maternal serum during the first trimester of pregnancy indicates that the synthesis and secretion of these proteins are closely related and may be regulated through a common mechanism. The qualitative patterns of the concentrations of each of these proteins in serum during this period closely resemble the logarithmic increase of the estimated trophoblastic mass. This suggests that, on a per cell basis, the secretory rate of each of these proteins is relatively constant during early pregnancy.

The concentrations of hPL and PSBG remain significantly correlated in individual samples of serum

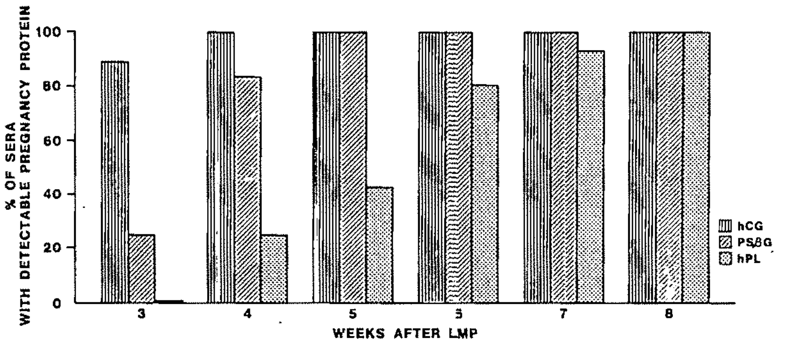

Fig. 8. Frequency of detection of hCG, PSBG, and hPL in the sera of early pregnancy. *LMP*, Last menstrual period.

during the second and third trimesters of gestation, and the pattern of secretion of these proteins continues to follow the growth of the estimated trophoblastic mass. However, during the second and third trimesters, there are substantial quantitative differences in the maternal levels of hPL and PSBG. The fortyfold greater concentration of PSBG may be related to a greater secretory rate by the syncytiotrophoblast of PSBG relative to hPL. Alternatively, the different concentrations of these two proteins in serum may reflect the longer serum half-life of PSBG (21.5 to 60 hours)[14, 32–34] in comparison to that of hPL (14 to 29 minutes).[4, 5, 35] Differences in the volume of distribution and metabolic clearance rates of these proteins may also account for the observed quantitative differences in their concentrations in serum.

Several investigators have examined the relationship between the concentrations of hPL and PSBG in sera obtained from women during the third trimester.[11–14, 36] Significant positive correlations have been found by Gordon and co-workers[13] and Schultz-Larsen,[14] in agreement with our findings. However, Lin and associates[11] and Towler and associates[12, 36] were unable to demonstrate a significant relationship, which possibly reflects the small number of patients studied or the statistical methods employed. As noted above, we found that the concentrations of proteins at each time interval more closely fit a log-normal than a normal distribution, and, therefore, we performed statistical analysis after logarithmic transformation or with nonparametric statistics.

In contrast to the significant correlation between hPL and PSBG during the last two trimesters of pregnancy, no significant correlation was found between hCG and hPL during the second trimester or between hCG and PSBG during the second or third trimesters. It is unlikely that this dichotomy is the result of differences in the half-life (5 to 40 hours),[37–40] volume of distribution, or metabolic clearance rate of hCG relative to the other two proteins, since studies of the qualitative pattern of the concentration of hCG in maternal blood or urine during pregnancy have consistently shown an increase during the first trimester, with peak concentrations between 8 and 12 weeks after the last menstrual period, followed by a decrease during the second trimester.[2] This occurs without a significant change in the metabolic clearance rate of hCG in any time interval during pregnancy.[41] Thus, it is evident that, after the first trimester, the mechanisms which control the secretion of hCG by the syncytiotrophoblast are different from those which control the secretion of hPL and PSBG.

Table II. Correlations between mean concentrations of hCG, hPL, and PSBG in maternal serum and estimated trophoblastic mass during each trimester

Protein	Trimester		
	First	*Second*	*Third*
hCG	0.98*	−0.62	0.51
hPL	0.87†	0.80‡	0.88†
PSBG	0.96*	0.84†	0.80†

*$p < 0.001$.
†$p < 0.01$.
‡$p < 0.05$.

Whether the decreased synthesis and secretion of hCG during the last two trimesters relative to the first trimester represent a change in the expression of the hCG genome at the nuclear level or modulation of gene expression at a cytoplasmic locus is not known. In vitro studies with placental ribosomes incubated in cell-free systems have demonstrated increased synthesis of hCG from first-trimester placental ribosomes relative to ribosomes prepared from term placentas, whereas the opposite has been found for hPL.[42, 43] However, the numbers of hPL genes per haploid genome were found to be the same in term and first-trimester tissue, thus suggesting that the enhanced synthesis of hPL messenger RNA as pregnancy progresses is the result of a transcriptional activation rather then activation of the hPL gene.[44] In addition, studies with placental explant cultures have shown a dichotomous secretory response of hPL and hCG after exposure to catecholamines, estriol, and cyclic adenosine monophosphate.[45, 46] Golander and co-worker[47] found that the secretion of hCG by term placental explants into the culture medium decreased between the first and second days, and then increased by an average of 780% from the second to fourth days. In contrast, the secretion of hPL was maximal on the first day, decreased by 83.5% on the second day, and remained constant over the next 2 days. Since no increase in the syncytiotrophoblastic mass was observed during the duration of the experiments, these investigators suggested that the increased synthesis of hCG was the result of the disappearance over time of an inhibitory factor present in the explants at the beginning of the culturing.[47] Thus, the data available from the in vitro studies provide support for the intracytoplasmic modulation of the secretion of hCG and hPL. Therefore, the qualitative differences in the secretory patterns of hCG and hPL during pregnancy may reflect inhibitory influences acting at the cytoplasmic level on the synthesis and secretion of hCG during the second and third trimesters.

The number of patients studied during early pregnancy allowed us to evaluate the measurement of hCG, hPL, and PSBG as a tool useful in the diagnosis of early pregnancy. Despite the localization by immunohistochemical techniques of all three proteins to the trophoblast of early gestation,[15–18] the sensitivity of the currently available radioimmunoassays of hPL has been found to be insufficient for detecting the quantities of this hormone in maternal serum before the first missed menses. Although PSBG has been detected in maternal serum as early as 7 days after ovulation, and 10 days after artificial insemination,[9, 10] we found that the measurements of PSBG were not so reliable for the diagnosis of pregnancy as were determinations of hCG by the beta subunit radioimmunoassay during the first 2 weeks after presumed conception.

We greatly appreciate the technical expertise of Ms. Jill McCready and Deborah Dare and the statistical consultation of Alan Forsythe, Ph.D. The reagents used for the radioimmunoassay of hCG and hPL were provided by the Hormone Distribution Officer, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland.

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Pure fetal blood from the umbilical cord obtained at fetoscopy: Experience with 125 consecutive cases

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With experience, and with the use of a 30-degree forward-oblique viewing telescope, sampling from one of the vessels of the umbilical cord produced at least 0.5 ml of pure fetal blood in 97% of the cases between 14 and 24 weeks' gestation; by aspiration through a short-tipped, 27-gauge, specially constructed needle, adequate samples were ensured. The technique used overcomes the problems previously encountered with sampling from an anteriorly situated placenta, and offers considerable potential for investigating intrauterine physiologic and pharmacologic features, as well as providing excellent specimens for diagnostic purposes. With this method, the calculated optimum time for fetoscopy and sampling of fetal blood is 16 to 18 weeks' gestation. (AM. J. OBSTET. GYNECOL. 138:1214, 1980.)

WITH THE DEVELOPMENT of sonar and fibreoptic endoscopic equipment, efforts have been made over the past 6 years to obtain blood from the fetus in utero during the second trimester of pregnancy, with a view to diagnosing inherited conditions.[1-3] The need for such an investigation is considerable when the magnitude of the distress caused by one condition, thalassemia major,[4] is contemplated. With the progress already achieved, greater efforts are now being made to obtain samples from an increasing number of transmitted diseases, including hemophilia, Duchenne's muscular dystrophy, and galactosemia.[5, 6]

When the placenta is situated posteriorly, a needle can be guided under direct vision with the use of a fetoscope into a major vessel on the surface of the placenta to obtain fetal blood. However, access to the anterior placenta is technically difficult with the use of a rigid fetoscope, and blind needling of the placenta has produced samples of fetal blood mixed with amniotic fluid or maternal blood.[7, 8] These samples are adequate for determining cell-transmitted disorders, such as the hemoglobinopathies, but the laboratory procedures required are demanding. For inherited diseases diagnosed by analysis of plasma or serum, such samples are inadequate. Therefore, a technique of fetoscopy and sampling of blood has been investigated with the primary object of reliably obtaining uncontaminated fetal blood for diagnostic purposes.

Patients and methods

One hundred twenty-five women admitted for therapeutic abortion within the terms of the 1967 Abortion Act were investigated; all gave informed, signed consent to be examined. Previous approval for the study had been granted by the Ethics Committee of the local hospital. Table I lists the gestation of the pregnancies studied.

In the majority of cases after admission, with the patient's bladder empty, an ultrasonic examination of the uterus was made by means of a Toshiba real-time scanner to assess pregnancy size and to localize the placenta; the site of entry into the amniotic cavity, with avoidance of the bulk of the placenta, was marked on the abdominal wall. One to 2 hours prior to operation, 15 mg of papaveretum was given intramuscularly as premedication, and 5 mg of diazepam was given intravenously immediately prior to fetoscopy. With the patient's bladder empty, 7 to 10 ml of 1% Xylocaine in epinephrine solution was injected under aseptic conditions into the anterior abdominal wall at the designated site, with infiltration extending into the myometrium.

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Supported by a grant from Oxford Hospitals Service Development Trust for purchase of fetoscopic equipment.

Received for publication April 3, 1980.

Revised and accepted August 8, 1980.

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Table I. Success rates according to gestation in obtaining pure fetal blood at fetoscopy

Instrument	*Cases*	*Gestation (wk)*		
		11-13	*14-17*	*18-24*
0° Angle telescope	*Cases 1-20:*			
	N	–	14	6
	Success	–	4 (29%)	2 (33%)
	Cases 21-46:			
	N	5	14	7
	Success	3 (60%)	9 (64%)	3 (43%)
30° Angle telescope	*Cases 47-125:*			
	N	8	38	33
	Success	5 (62%)	37 (97.4%)	32 (96.7%)

Success = aspiration of at least 0.5 ml of pure fetal blood.

All examinations were performed with a Storz fetoscope (26300C), with outer dimensions of 3.3 by 4.5 mm and incorporating a site channel to accommodate a 22 gauge aspiration needle. Initially, a Hopkins miniature "straightforward 0°" telescope (27018A) was used, but since during the course of the study its use became associated with technical difficulties in obtaining fetal blood, it was replaced by a Hopkins miniature "forward-oblique 30°" telescope (27018A) for the last 79 cases. After insertion of the fetoscope, orientation within the amniotic cavity was made, with identification of fetal landmarks and the placental site; wherever possible, insertion of the umbilical cord into the chorionic plate was localized. Needle aspiration from one of the three vessels of the umbilical cord was attempted at a convenient point along the cord's length, ideally near its insertion into the chorionic plate or at the fetal end, the aspiration was accomplished while ensuring that the needle remained within the lumen of the vessel. To facilitate the collection of blood, a modification of the aspirating needle was made: a 22 gauge needle, 320 mm long, that incorporated a 27 gauge aspirating tip, 3 mm long, was constructed (Fig. 1). In a few instances, aspiration of a major vessel on the placental surface was also performed.

The procedure was considered to be a success whenever at least 0.5 ml of uncontaminated fetal blood was collected into a standard 2 ml disposable plastic syringe, which was immediately anticoagulated with 10% of its volume with heparin; confirmation that the blood was of fetal origin was made by means of the acid elution technique of Kleihauer and associates.[9] After the examination, PGE_2 was instilled intra-amniotically to induce abortion.

Results

In all instances, apart from the discomfort felt with the injection of local anesthetic, patients experienced no further pain due to the procedure. In a few cases in which intravenous diazepam was omitted, the patient appeared to be sufficiently sedated with the premedication, but fetal activity observed with the fetoscope was excessive and caused some difficulty in obtaining adequate views of fetal landmarks and samples of blood, a problem which was more marked with gestations of 18 weeks and more. There were no maternal complications as a direct result of the fetoscopic examination either immediately or within the subsequent 6 weeks.

Table I illustrates the success in obtaining pure fetal blood according to gestational grouping. An arbitrary subdivision of the first 46 consecutive cases using the 0° telescope has been made. The last 79 cases studied with the use of the 30° telescope have been analyzed separately: for the 74 successful attempts, more than 1.0 ml of pure blood was obtained in 93% of the cases and more than 3 ml in 72%.

Of the 25 failures among the first 46 cases, three were due to failure to enter the amniotic sac, twice at 11 weeks' gestation and once at 14 weeks; 15 failures were due to technical difficulties, three were due to troublesome bleeding from the site of entry of the fetoscope through the placenta that had not been previously localized, two were due to cloudy amniotic fluid after a previous diagnostic amniocentesis, and one was due to bleeding from the site of fetal blood sampling. Three of the five failures among the last 79 cases in which the 30° telescope was used were at 11 to 13 weeks' gestation and were due to profuse bleeding from the attempted sampling site, almost certainly leading to fetal exsanguination; excessive bleeding from the fetoscopy entry site was the cause of failure in one, whereas in the other the failure was due to densely discolored liquor after a diagnostic amniocentesis performed 2 weeks previously. With the exception of the three cases mentioned, bleeding from the sampling site in the umbilical cord ceased within a few seconds.

Of 10 cases in which blood was sampled from a major

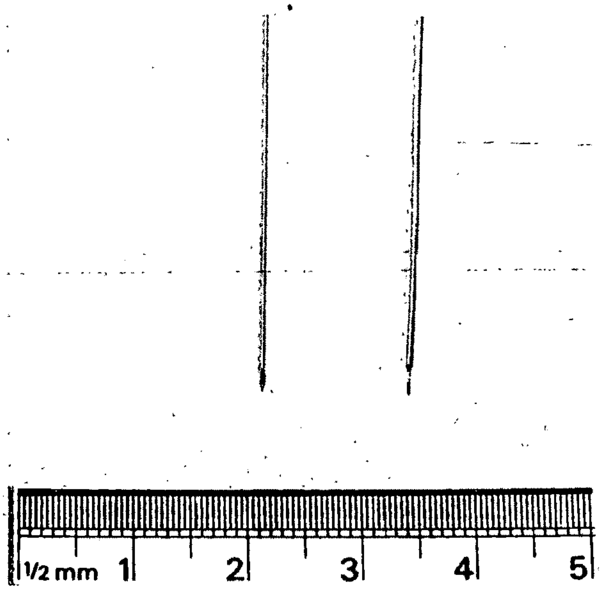

Fig. 1. Needle tip for aspirating fetal blood. Example on the left is the modification with 22 gauge shaft and 27 gauge aspirating tip, 3 mm long.

vessel on the placental surface, pure fetal blood was obtained in four, mixed fetal and maternal blood in two, and pure maternal blood in four.

There was difficulty in obtaining adequate views of the fetus, cord, and placenta in 16 cases. In five, this was primarily due to the fact that the patient had undergone a diagnostic amniocentesis 1 to 2 weeks previously, with the liquor being either very turbid or thickly stained from previous bleeding. In 11 cases, the obscured view resulted from bleeding at the site of entry of the fetoscope into the uterus; three of these cases occurred during the first 20 patients examined and were totally due to technical problems, whereas six of the other eight cases occurred when the placenta had not been localized ultrasonically and was situated anteriorly. Of 97 patients in whom placental localization had been performed prior to fetoscopy and confirmed at examination, the placenta was anterior in 66. With care in siting the entry into the amniotic sac with prior ultrasonic guidance so as to avoid the main mass of placenta, this feature was not associated with a failure to obtain pure fetal blood.

Comment

We concur with others that fetoscopy and the sampling of fetal blood require considerable practice and expertise.[10] Our experience has been that 20 to 40 attempts are necessary before success can be reliably achieved. With the use of the Storz forward-oblique 30° viewing telescope, the visual field was not significantly reduced and the benefits were considerable for aspiration of fetal blood. Although none of the patients was distressed by the procedure with the sedation provided, the intravenous injection of diazepam immediately prior to fetoscopy was particularly valuable in reducing fetal activity and, thus, in facilitating inspection of fetal anatomy and aspiration of fetal blood.

Obtaining samples of fetal blood not contaminated with maternal blood or amniotic fluid has the great advantage of reducing required laboratory procedures. Examining subjects for thalassemia, Fairweather and associates[8] analyzed blood obtained by blind needling of the anterior placenta and direct aspiration at fetoscopy from a posterior placental vessel, and succeeded in getting samples with 100% fetal cells in only four of 22 cases; 11 of the 22 cases required more than one attempt. A Coulter particle-size analyzer was used for the immediate differentiation of fetal from maternal red cells, with the patients having previously undergone transfusion to suppress activity of maternal bone marrow. Similar results were obtained by Golbus and associates[11] using placental needling in 28 cases when 13 samples contained 75% to 100% fetal cells. Using the particle-size analyzer and subsequent acid elution technique for confirmation, Rodeck and Campbell[12] succeeded in getting samples with 100% fetal cells by direct aspiration at fetoscopy from a placental surface vessel near the cord's site of insertion in 40 of 48 cases at 16 to 22 weeks' gestation; 22 samples were pure fetal blood not contaminated by amniotic fluid. With experience, using the 30° angle telescope and aspirating blood directly from one of the three umbilical cord vessels, we obtained pure fetal blood in 97% of the cases over the same gestational period. The specially designed needle permitted a minute puncture site in the vessel wall, thus reducing subsequent bleeding on withdrawal, provided a guide to the depth of penetration into the vessel, and allowed for an adequate flow of blood to prevent intraneedle clotting. For the diagnosis of hemophilia, the interpretation of results obtained for samples contaminated with amniotic fluid requires considerable caution: the same caution can be dismissed when pure samples are obtained.

The benefits of localizing the placenta by scanning with real-time ultrasound before fetoscopy are obvious: of the last 57 cases examined, troublesome bleeding into the amniotic sac subsequent to instrumentation occurred in eight cases, in six of which localization had not been performed. The suggestion was previously made that, when the placenta is anterior, the sampling of fetal blood with a rigid instrument is unsuccessful;

thus, resort to blind placental needling has been advocated.[8] In the present study, the bulk of the placenta was situated anteriorly in 67.5% of the cases, a figure similar to that reported by other workers.[6, 8, 12] With aspiration from the umbilical cord, the technical difficulties are largely circumvented.

Unlike Rodeck and Campbell,[12] we did not find that success in obtaining samples of pure fetal blood is reduced below 18 weeks' gestation. We have been impressed by the advantages of performing fetoscopy between 14 and 17 weeks in order to view different parts of the fetal anatomy; endeavors at fetoscopy earlier than that gestational age were, however, less rewarding. When the procedure is performed before 18 weeks' gestation, more time is allowed to induce abortion in those cases in which the suspected inherited disorder is confirmed. However, we are uncertain of the volume of blood that can safely be removed from the fetoplacental circulation. Morris and associates[13] have calculated that the fetoplacental blood volume is 16.2 ± 2.0 ml per 100 grams of fetal weight at 16 to 22 weeks' gestation. Since the mean fetal weight at 14 weeks is 50 grams, and rises to 100 grams at 16 weeks and 200 grams by 18 weeks,[14] it seems prudent to suggest that 16 weeks might be the earliest gestational age at which to attempt sampling of fetal blood, when 1 ml of fetal blood might be removed without compromising the fetus. It is difficult to estimate the volume of leaking blood after withdrawal of the needle from the fetal vessel, but previous observation has been that such bleeding usually ceases within a few seconds.[1, 10, 11, 15] This was the experience in the present series, with the exception of three cases at 11 to 13 weeks' gestation.

Since all the pregnancies examined were therapeutically aborted, the effect of this technique of fetoscopy and sampling in the short and long term cannot be stated. Fetal heart sounds were heard for at least 5 hours in five of six patients at 17 to 19 weeks' gestation after collection of between 4 and 6 ml of fetal blood, thus suggesting that at this gestational age the fetus is capable of compensating for this large volume of lost blood. Similar observations were made by Hobbins and Mahoney[15] in 14 patients in whom much smaller volumes of blood were removed. Until large numbers of continuing pregnancies have been examined by the fetoscopic method of obtaining pure fetal blood, the precise fetal wastage rate cannot be known; it is reasonable to surmise that it is likely to be in the same range as the 3% to 12% reported by others.[8, 12] However, the outer dimensions of the instrument used in the present study are considerably larger than those of the instruments used by most other workers. Although the larger instrument provides a greater field of vision and probably shorter sampling times, it is possible that post-fetoscopy abortions may result more often. Until large numbers of continuing pregnancies have been examined with the Storz fetoscope, the pregnancy loss rate cannot be stated.

The results obtained in the present series indicate that uncontaminated samples of fetal blood can be reliably obtained by umbilical vasopuncture between 14 and 24 weeks' gestation, even when the main mass of placenta is situated anteriorly. With the relatively large samples of blood obtained, this technique expands the possibilities for further research into inherited diseases, fetomaternal physiologic features, and the pharmacokinetics of drugs administered during pregnancy.

We wish to thank Sister Anna Fry for her expert nursing care, Mrs. Beryl Moffat, Department of Haematology, Dr. Jean Keeling, Department of Paediatric Pathology, and the staff of the Radiology and Ultrasonography Department, John Radcliffe Hospital. Our thanks are also due to Professor A. C. Turnbull for advice and encouragement in pursuing this study. The modified aspiration needles were manufactured with the help of Needle Industries, Redditch, England.

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COMMUNICATIONS IN BRIEF

This section is suitable for reporting results of therapeutic trials, descriptions of new procedures or instruments, and case reports which illustrate a principle. Reports should be limited to seven hundred words and two references. Use of an illustration or table requires a proportionate reduction in total words.

Group B streptococcus and puerperal sepsis

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IMMEDIATE PUERPERAL SEPSIS has long been associated with group A, beta-hemolytic streptococcal infection. Group A streptococcal infection, if not recognized early, usually follows a fulminant course. Lancefield group B, beta-hemolytic streptococcus *(Streptococcus agalactiae)* is not recognized as a major cause of maternal morbidity, even though 25% of pregnant women may be colonized by this bacterium. Hood and associates[1] reported maternal amnionitis and endometritis secondary to group B, beta-hemolytic streptococcal (GBBS) infection which resolved shortly after delivery. Finn and Holden[2] reported six cases of GBBS puerperal sepsis; three of these six cases were associated with prolonged rupture of amniotic membranes.

This report concerns 14 cases of GBBS puerperal infection which occurred on the Louisiana State University obstetric service at Charity Hospital in New Orleans. The colonization rate on the LSU obstetric service is 23% (unpublished data). Over a 6-month period, 14 cases of postpartum endomyometritis secondary to GBBS infection occurred, six with associated bacteremia. The unique features of these cases were: (1) All patients were delivered by cesarean section; (2) the average gravidity was 1.8; (3) the average age was 21; (4) the patients had ruptured membranes for an average of 14.4 hours with a range of 0 to 40 hours; (5) the average duration of operation was 1.23 hours; (6) all had temperature elevation shortly after delivery, occurring on the average within 17.3 hours, with the average temperature being 39.1° C; (7) all patients developed a tachycardia; (8) all developed an exquisitely tender uterus which coincided with an elevation in temperature.

Specimens were obtained for aerobic and anaerobic bacterial culture from the endometrial cavity via a sterile swab passed through the endocervical canal. In addition, an aspirate from the posterior cul-de-sac as well as three separate samples of venous blood for culture was obtained. All patients had GBBS recovered from at least one site, with the endometrium being the most common site. A total of five patients yielded only GBBS, with the remainder of the patients having polymicrobial infections consisting of aerobic and anaerobic bacteria.

Patients with bacteremia presented with an accompanying endomyometritis, shaking chills, and a temperature spike of 39° C within 10.6 hours of delivery. One primigravid patient with pre-eclampsia, who was delivered by cesarean section because of cephalopelvic disproportion, became febrile 12 hours after delivery. Temperature rose to 40° C; blood pressure was 220/150 mm Hg; pulse was 160 bpm, and respirations were 40 per minute. She became oliguric and had severe uterine tenderness. Over a 2-day period a total of six blood cultures were obtained, and each grew GBBS. In addition, the endometrial specimen yielded a pure culture of GBBS.

GBBS infection thus poses a serious threat to the mother as well as the neonate. Maternal postpartum sepsis secondary to GBBS infection occurs rapidly, usually within the first 24 hours of delivery, and the patient may become seriously ill within a short period of time. Patients with prolonged rupture of membranes and dysfunctional labors which result in cesarean section should be considered at risk for GBBS infection. Appropriate cultures for aerobic and anaerobic bacteria

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should be obtained at operation. For example, an aspirate of amniotic fluid and a swab passed over the endometrium may be obtained without difficulty.

GBBS infection is similar to group A beta-hemolytic streptococcus infection in that the signs and symptoms of disease occur early in the puerperium, followed by rapid deterioration of the patient's condition if vigorous treatment is not instituted. Therefore, patients exhibiting signs of early infection—temperature elevation above 38.5° C, tachycardia, and an exquisitely tender uterus—within the first 24 hours of delivery should be considered to be infected and GBBS should be suspected as the etiologic agent. Appropriate specimens should be obtained for culturing aerobic and anaerobic bacteria. Antibiotic therapy should be instituted immediately.

GBBS is sensitive to penicillin, and since obstetric infections are often polymicrobial, a broad-spectrum antibiotic such as Ticarcillin or a cephalosporin such as Cefoxitin should be administered. Both Ticarcillin and Cefoxitin provide suitable anaerobic coverage including *Bacteroides fragilis.* These antibiotics also provide adequate coverage against *E. coli.* It is important that antibiotic therapy be started early to prevent serious infections such as abscess formation and endocarditis.

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Modified technique for monitoring fetal heart rate during intrauterine transfusion

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SEVERAL AUTHORS have attempted to show the predictive value of the fetal heart rate response to intrauterine transfusion (IUT).[1, 2] Analysis of these responses has been plagued by limitations of external

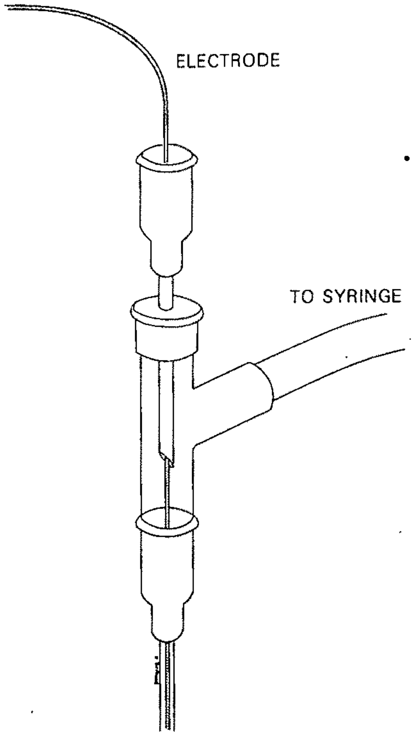

Fig. 1. Details of insertion of electrode. The diaphragm of the Y-connector is perforated with a 3 cm needle through which the electrode wire is inserted and advanced through the transfusion catheter.

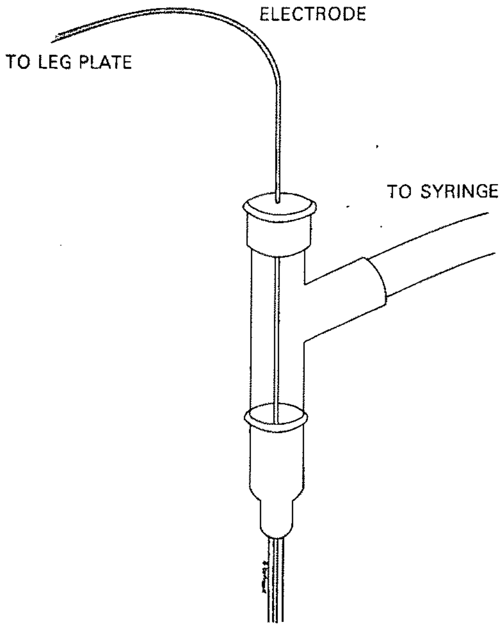

Fig. 2. The 3 cm needle has been removed, leaving the electrode in place. The diaphragm of the Y-connector holds the electrode wire in place and prevents reflux along the wire.

Supported in part by The Ariel Kaare Røsholt Weathers-Lowin Medical Research Foundation.

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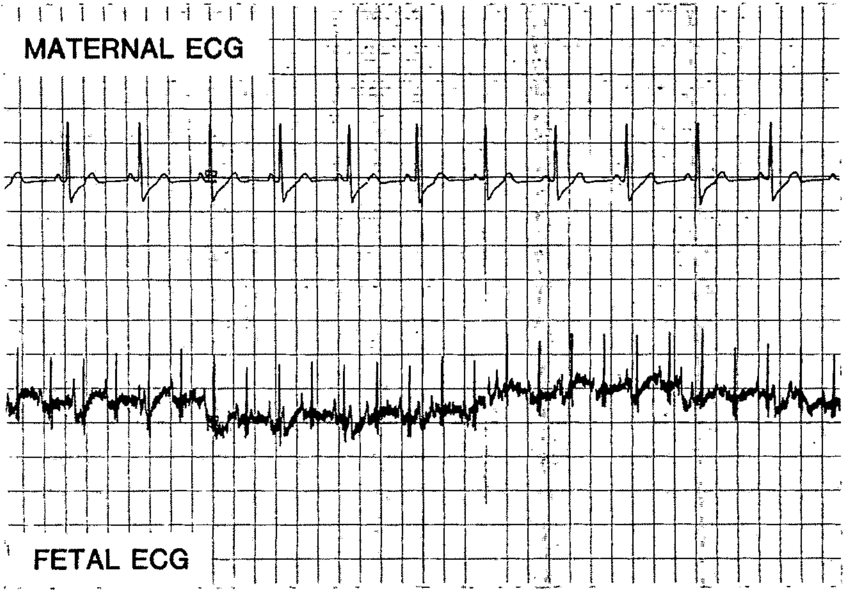

Fig. 3. Simultaneous maternal and fetal ECG during IUT. The maternal ECG was obtained with precordial leads. The fetal ECG was obtained via the intra-abdominal (fetal) electrode wire, as described in the text.

transducers (ultrasound, phonocardiograph, abdominal electrocardiograph) which do not permit adequate assessment of fetal heart rate variability. Moreover, external devices are clumsy and cumbersome and difficult to maintain in sterile condition. At the extremes of heart rate, ultrasound devices also tend to double or half-count the rate.[3] Attempts to obtain the fetal electrocardiogram directly from the transfusion needle or a stylet inserted through an epidural catheter have met with variable success.[1, 2] With the former, resolution of the signal is poor, whereas with the latter, monitoring during various parts of the procedure is limited.

In 1968, Crosby[4] described a catheter/electrode in which a wire electrode was mechanically attached to the tubing used for the IUT. This device, while effective, was not commercially available. In addition, prolonged recording required that the entire device be left in place.

In an effort to simplify the procedure and improve the resolution of fetal heart rate monitoring during transfusion, we developed a direct monitoring technique which appears to be safe and reliable and provides high-quality ECG tracings throughout the procedure. The purpose of this report is to describe this technique. An analysis of the data from these cases will be presented subsequently.

We perform IUT with the use of real-time ultrasonography.[5] The benefits of this technique include avoidance of the placenta at amniocentesis, decreased exposure to radiation, and no complications from intrauterine dye. In place of the time-honored Touhey needle, we use a 20 cm, 16 gauge, Teflon-sheathed needle catheter,* which is larger, more flexible, and less traumatic than the needle.

The abdomen is prepared and draped in the usual fashion, and the needle is inserted under real-time ultrasound guidance. The location of the tip of the needle can best be determined by the observation of turbulence after the injection of a small volume of saline solution through the catheter. Once the catheter is inserted into the fetus, the needle is removed and a Y-connector is attached to the Luer-Lok hub of the catheter. The diaphragm of the Y-connector is perforated with an 18-gauge, 3-cm needle through which a pediatric cardiac electrode pacing wire† is inserted (Fig 1). The wire, previously marked to 18 cm, is advanced to the mark, and the short needle is removed from the diaphragm (Fig. 2). The diaphragm effectively holds the wire in place and at the same time prevents any reflux along the wire.

The wire is connected to the positive pole of a fetal monitoring leg plate. The second terminal of the leg plate is connected to a standard surface electrode by an appropriate cable. The output of the fetal monitor is hooked up to an oscilloscope and/or strip chart recorder for display of the ECG along with the fetal heart rate.

*I.V. Cath 16G8, 6761, Becton, Dickinson & Co., Rutherford, New Jersey.

†Pediatric Cardiac Electrode 277933, Edward Weck Co. Inc., Research Triangle Park, North Carolina.

By means of this procedure, the fetus can be monitored during the administration of blood or dye or even during the removal of ascitic fluid. The quality of the fetal ECG is excellent, in the majority of cases the signal-to-noise (S/N) ratio has been greater than 6 (Fig. 3). We have generally removed the catheter immediately after the transfusion but have left the electrode in place for several hours. Thus far, there have been no failures or complications in 12 cases of IUT performed by this technique. The wire is thin and very flexible, and the bare end is trimmed short to prevent any possible perforation. The advantages of this technique include convenience, simplicity, and high-resolution tracings during the entire procedure. This technique facilitates the recording of fetal heart rate during IUT and perhaps other procedures, and may prove to be of diagnostic and prognostic usefulness.[6] This procedure may also be useful in experimental animals in which direct recordings of fetal heart rate can be obtained without hysterotomy.

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A study of ovarian cancer patients using a radioimmunoassay for human ovarian tumor-associated antigen OCA

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SINCE the prognosis for patients with ovarian cancer is dependent upon the stage at which the tumor is detected, earlier detection of ovarian tumors should lead to a dramatic improvement in the survival rate of patients. We previously reported the development of a competitive inhibition double-antibody radioimmunoassay for detecting nanogram amounts of human ovarian tumor-associated antigen OCA in plasma.[1] The positive level of the assay was determined to be equal to

Table I. Plasma OCA levels by stage of disease

Stage	*No. with positive OCA levels*	*Total No. of patients*	*% Positive*
I	21	27	78
II	40	54	74
III	45	57	79
IV	10	15	67
Total	116	153	76

Table II. Plasma OCA levels by pathologic type of tumor

Tumor	*No. with positive OCA levels*	*Total No. of patients*	*% Positive*
Serous cystadenocarcinoma	38	59	65
Mucinous cystadenocarcinoma	13	20	65
Endometrioid carcinoma	13	24	54
Adenocarcinoma	14	22	64
Others	5	13	38

Table III. Plasma OCA levels of patients with serous tumors by stage

Stage	*No. with positive OCA levels*	*Total No. of patients*	*% Positive*
I	9	11	82
II	11	13	85
III	12	19	63
IV	4	7	57

Table IV. Plasma OCA levels by pathologic type and differentiation of tumor

Tumor	*Well differentiated*	*Poorly differentiated*
Serous cystadenocarcinoma	12/19 (63)*	15/19 (79)
Mucinous cystadenocarcinoma	7/10 (70)	4/7 (57)
Endometrioid carcinoma	1/1 (100)	10/19 (53)
Adenocarcinoma	1/2 (50)	6/10 (60)
Total	21/32 (66)	35/55 (64)

*Number of patients with positive OCA levels/total number of patients (% positive).

Supported by Ontario Cancer Treatment and Research Foundation Project No. 324.

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or greater than 2.0 ng of OCA activity per milliliter of plasma. In a preliminary study in which the OCA assay was used,[1] 52% of the patients with ovarian cancer who were tested had positive plasma levels of OCA, as compared to 10% of patients with noncancerous gynecologic disease, 10% of pregnant patients, and 10% of apparently disease-free controls. The present study was undertaken in order to determine whether the OCA was specific for one type or one stage of ovarian tumor.

Samples of plasma from patients with ovarian cancer who were attending the Outpatient Clinic at the Princess Margaret Hospital, Toronto, Canada, were examined to determine the correlation between elevated plasma levels of OCA and tumor staging, tumor pathologic features, and degree of tumor differentiation. Samples of blood were taken from each patient on at least two separate visits to the clinic. In order to be included in this study, the plasma levels of OCA of each patient were required to be consistently negative or positive; those patients who had fluctuating levels of OCA were eliminated from the study.

Of 153 patients, 116 patients, or 76%, had positive plasma levels of OCA. When these same patients were grouped by stage of disease according to the staging system of the Union Internationale Contre le Cancer (Table I), the finding was that the level of OCA positivity did not significantly increase with increasing stage of disease. When 138 of these same patients were grouped according to pathologic type of tumor (Table II), there was no correlation ($p > 0.1$) between the level of OCA positivity and the pathologic type of tumor. When the 50 patients with serous tumors were grouped by stage of disease (UICC staging) (Table III), there was an apparent decrease in OCA positivity with increasing stage of disease, but this difference was not statistically significant ($P > 0.1$). When patients were grouped according to both pathologic type and degree of differentiation of the tumor (Table IV), there was no correlation between degree of differentiation and OCA positivity.

Since ovarian tumor–associated antigen OCA levels are elevated in early-stage (minimal) disease, and the antigen is present in the plasma of patients with ovarian cancer regardless of pathologic type and differentiation of the tumor, it may be possible to use the radioimmunoassay to detect circulating tumor antigen before clinical signs of disease are apparent.

We wish to thank Dr. R. S. Bush for submitting patient material and Mr. G. Hou for excellent technical assistance.

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Uterine rupture at term with vaginal prostaglandin E_2

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PROSTAGLANDIN E_2 (PGE_2) is a powerful oxytocic agent that reliably initiates labor, even in the presence of an "uninducible" cervix.[1] It is available in vaginal suppositories specifically indicated for the management of intrauterine fetal death (IUFD).[2] Although mild side effects are common with PGE_2 suppositories, serious complications are rare. The following case illustrates a particularly uncommon complication.

L. B., a 25-year-old, para 4-2-0-2-2 patient, with questionable gestational dates, presented to the labor room claiming loss of fetal movement since the evening before. The fetal heart beat could not be heard and no cardiac activity was discerned by real-time ultrasonography. Estimated fetal weight was 7 pounds and presentation was vertex. On vaginal examination the cervix was 20% effaced and 2 cm dilated. Station was −3. The membranes were ruptured. The internal monitor catheter was inserted, and one 20 mg PGE_2 suppository was placed deep in the vaginal fornix. Labor began within 1 hour and was characterized by polysystole without hypertonus (Fig. 1). With only the one suppository, the cervix was fully effaced in 6 hours and fully dilated in 7 hours. Delivery came shortly after full dilatation with spontaneous separation of the placenta. The stillborn infant as well as the placenta appeared grossly normal and appeared to be of term maturity. After delivery, the patient bled approximately 1,000 ml; bleeding slowed significantly after oxytocin administration. However, pelvic examination revealed a defect in the right lateral uterine wall. The patient was brought to the operating room where an exploratory operation was performed. Laparotomy revealed a large hematoma beneath the vesicouterine peritoneum, which dissected into both retroperitoneal spaces overlying the pelvic vessels to the level of the infundibulopelvic ligaments. Examination of the uterus revealed bilateral lower segment lacerations which included the uterine vessels. A bilateral hypogastric ligation, total abdominal hysterectomy, and left salpingo-oophorectomy were performed.

This case demonstrates uterine rupture despite the absence of hypertonicity with PGE_2. The case is unusual in several respects. Uterine rupture secondary to PGE_2 has been described in one case report of a second-trimester abortion,[1] but its use in over 240 women with IUFD was not accompanied by even one such complication.[2] In addition, only one suppository

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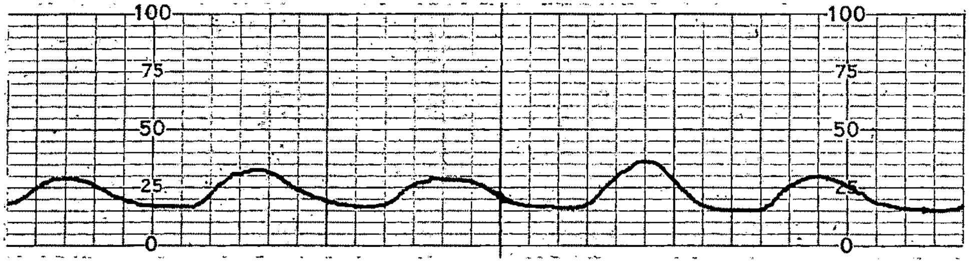

Fig. 1. Uterine polysystoly on internal pressure monitoring.

was used during our patient's 8-hour labor rather than an every- 3-hour dose as usually recommended. The uterine rupture was caused by the polysytolic labor patterns. Thus, even though PGE_2 suppositories can be extremely useful for the management of IUFD, this potentially catastrophic event is a rare but possible complication.

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A prolapsed umbilical cord into the abdominal cavity in a woman with a previous cesarean section

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WOMEN with previous cesarean sections performed through a low transverse uterine incision for a nonrepetitive indication (i.e., breech, fetal distress) may be candidates for attempted vaginal delivery at LAC/USC Women's Hospital. They are closely monitored but otherwise are managed as normal multiparous patients. We report here an unusual presentation of uterine rupture.

A 31-year-old woman, gravida 4, para 2, abortions 1, was admitted at term with ruptured membranes for 3 hours and no uterine contractions. Her first pregnancy resulted in a normal vaginal delivery of an 8 pound infant. Two years before the current pregnancy, she had a primary low transverse cesarean section while not in labor for "postmaturity."

Examination on admission confirmed the ruptured membranes and showed the cervix to be 50% effaced and 2 cm dilated with a vertex presentation at the −3 station. Estimated fetal weight was 3,800 gm. A transcervical intrauterine pressure catheter was placed posteriorly, and a fetal electrode was attached to the scalp. Oxytocin was started and adequate labor was established with a resting tone of 15 to 20 mm Hg and contractions every 2 to 4 minutes, reaching 25 to 40 mm Hg above the baseline. Within 8 hours the cervix had dilated to 7 cm and the head had descended to the 0 station. The baseline fetal heart rate was 140 to 150 bpm with good variability and few periodic changes. During the next hour the cervix dilated to 9 cm and the head descended to the +2 station, but increasing variable decelerations occurred and the baseline fetal heart rate gradually climbed to 160 bpm. There was no significant change in resting uterine pressure or the contraction pattern. At no time did the mother complain of undue pain and the vital signs remained stable. A Foley catheter inserted prior to delivery showed clear urine. A 5-minute unremitting deceleration to 60 bpm resulted in an emergency cesarean section. When the peritoneal cavity was entered, a 6 cm dehiscence of the lower uterine incision was discovered, through which protruded the top third of the infant's head and approximately 12 inches of the umbilical cord. A female infant weighing 4,140 gm was delivered, with Apgar scores of 0 at 1 minute and 3 at 5 minutes. Cord gas values were compatible with acute respiratory acidosis: arterial pH, 6.67; Pco_2, 129 mm Hg; bicarbonate, 14 mEq/L; base deficit, −20; venous pH, 7.20; Pco_2, 49 mm Hg; bicarbonate, 19 mEq/L; base deficit, −8. The uterine incision was bleeding minimally and was repaired without difficulty. Both infant and mother were subsequently discharged without further complications.

It is generally agreed that scars in the noncontractive lower uterine segment, when compared to vertical fundal incisions, are much less likely to rupture prior to labor. Some authors[1] argue that during labor the lower segment scars may offer no greater protection than classical incisions. The maternal course of our patient was not unusual for a rupture of a previous scar in that she was essentially asymptomatic and lacked the overt signs of sudden abdominal pain, bloody urine, decreased uterine contractions, and shock. The use of oxytocin in patients with previous uterine scars is not

Supported by National Institutes of Health Grant No. HD07086-05.

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generally advocated but is under study at our institution. The unusual aspect of this case was not the uterine rupture but the clinical presentation, which was fetal distress due to compression of the prolapsed umbilical cord. In patients with previous uterine scars variable decelerations may have a more ominous meaning.

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Severe hypokalemia and acidosis: A potential complication of beta-adrenergic treatment

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BETA-ADRENERGIC AGENTS, such as terbutaline, isoxsuprine, ritodrine, and Salbutamol, are being widely used to inhibit premature labor. Reported maternal side effects of this class of drugs include hypotension, pulmonary edema, cardiac arrhythmias, and hyperglycemia[1]; fetal/infant side effects include tachycardia, hypoglycemia, and hypocalcemia.[2] Although mild hypokalemia has been reported in the British literature as a side effect of Salbutamol,[3] hypokalemia has not been reported as a complication of beta-adrenergic agents in the American literature and is not mentioned in the terbutaline product insert. Our purpose here is to report an episode of severe hypokalemia, accompanied by acidosis, developing in a normal pregnant patient treated with terbutaline and betamethasone.

A healthy 22-year-old primigravid patient, receiving no medication, presented at 32 weeks' gestation in premature labor. The cervix was 3 cm dilated and 100% effaced. After discussion of the known risks and options, betamethasone, 12 mg, was administered intramuscularly and terbutaline was started intravenously, the dose being increased every 10 minutes until the patient was receiving 25 μg/min. After 1 hour, the contractions stopped; the infusion was decreased to 15 μg/min and continued for the next 4 hours. This resulted in a total dose of terbutaline of 6,300 μg in 0.5 L of 5% dextrose in water over the first 6 hours, at which time sodium = 131 mEq/L, chloride = 104 mEq/L, potassium = 2.2 mEq/L, total carbon dioxide content = 12 mEq/L, and glucose = 452 mg/dl.

Active labor then resumed and could not be suppressed by 2 additional hours of terbutaline (25 μg/min). Electronic fetal monitoring continued to show only a persistent tachycardia of 180 bpm. Because of a footling breech presentation, the patient was delivered abdominally with epidural anesthesia. During delivery and the first 36 hours postoperatively, she received 1.5 L of lactated Ringer's solution in 5% dextrose without supplemental potassium. Twelve hours following operation, maternal serum sodium = 135 mEq/L, chloride = 137 mEq/L, potassium = 3.1 mEq/L, total carbon dioxide content = 21 mEq/L, and glucose = 112 mg/dl; at 36 hours, sodium = 137 mEq/L, chloride = 105 mEq/L, potassium = 4.2 mEq/L, total carbon dioxide content = 24 mEq/L, and glucose = 41 mg/dl. The mother's postpartum course was normal.

The 1,760 gm male infant had Apgar scores of 1 and 6 at 1 and 5 minutes, respectively. He was treated with oxygen by mask and sodium bicarbonate, 2 mEq/kg, intravenously. At delivery, the serum glucose level = 248 mg/dl. Two hours later, an arterial blood gas sample showed pH = 7.18, Po_2 = 85 mm Hg, Pco_2 = 52 mm Hg, and standard bicarbonate = 19.4 mM/L. At 12 hours of age, the infant's serum sodium = 133 mEq/L, chloride = 108 mEq/L, potassium = 4.2 mEq/L, and total carbon dioxide = 19 mEq/L. He did not become hypoglycemic and subsequently did well.

This patient's course was marked by unanticipated severe hypokalemia (potassium = 2.2 mEq/L) during a standard treatment protocol for inhibition of premature labor.[1] Hypokalemia can occur with long-term steroid treatment but would be unlikely with the single dose of betamethasone administered here. It was more likely a complication of treatment with terbutaline, for at least three reasons: (1) Mild hypokalemia secondary to treatment with another beta-adrenergic agent, Salbutamol, has been previously reported.[3] (2) A likely mechanism for hypokalemia would be the well-recognized hyperglycemia and hyperinsulinemia stimulated by beta-adrenergic agents. As glucose moves intracellularly, potassium is similarly shifted from the extracellular to the intracellular space, in exchange for hydrogen ion. Indeed, in the current case, the maternal total carbon dioxide content of 12 mEq/L was suggestive of metabolic acidosis and the infant was clearly acidotic. Maternal/fetal acidosis has been previously associated with beta-adrenergic treatment, although the mechanism suggested was an increase in lactate induced by these agents, rather than an intracellular shift of potassium.[1] (3) Further support for considering hypokalemia to be a complication of terbutaline treatment is provided by the fact that several well-recognized maternal/fetal/infant complications of beta-adrenergic therapy may be explained on this basis. These complications might include cardiac arrhythmias, paralytic ileus, and maternal tremors, in addition to acidosis.

Supported in part by United States Public Health Service Grants 5M01-RR00210 and 1P50 HD 11089.

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Therefore, it would appear that physicians using beta-adrenergic agents should be aware of their potential for producing severe hypokalemia, accompanied by acidosis. At present, we recommend monitoring serum electrolyte and glucose levels prior to and during therapy. Since we began obtaining these studies, we have observed several similarly treated patients with serum potassium levels in the 2 to 3 mEq/L range. Whether administering supplemental potassium or withholding exogenous glucose and/or lactate would be valuable in avoiding these problems remains to be seen.

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Tularemia immunization in early pregnancy

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TULAREMIA is a bacterial infectious disease caused by *Francisella tularensis* and communicated to man via arthropod and animal vectors or by ingestion of contaminated food and water. The most common manifestations include ulcerative lesions of the skin and mucous membranes accompanied by localized lymphadenopathy, fever, and occasionally pneumonia. The mortality rate in untreated cases is 6% to 7% whereas with effective antibiotic therapy death is rare. Hunters and outdoorsmen exposed to infected rabbits, squirrels, foxes, muskrat, and deer are a high-risk population. In addition, laboratory research personnel working with *F. tularensis* form a high-risk group.

The last reported case in the English literature of tularemia in pregnancy that we have been able to find was in 1936.[1] In view of this apparently low incidence, it appears that the obstetrician may more likely encounter the disease in reference to vaccination. Although the live attenuated vaccine has been available since 1970, no data with regard to the safety of this vaccination during pregnancy have been reported. The following is a report of a patient inadvertently immunized at the calculated time of conception.

The patient was a 23-year-old laboratory technician. She received live attenuated tularemia vaccine for employment purposes 2 weeks after her last menstrual period. A urine pregnancy test was positive at 6 weeks beyond her last menstrual period. The prenatal course was unremarkable except for tularemia agglutination titers which were 1:80 throughout the pregnancy.

At a gestational age of 39 weeks and 4 days labor occurred after spontaneous rupture of the membranes. On presentation to the hospital, meconium-stained amniotic fluid was noted. Internal electronic fetal heart rate monitoring indicated normal findings. The patient was delivered of a 3,890 gram male infant via low forceps. There was no evidence of lymphadenopathy, mucocutaneous ulcerative lesions, or congenital pneumonia. All bacterial cultures including blood cultures were negative. Maternal serum revealed a 1:80 tularemia agglutination titer while cord serum revealed a 1:20 titer.* One-year follow-up of the infant showed normal development.

Immunization with live attenuated agents just prior to or during pregnancy is a subject that has received much attention during the past decade. It is generally recommended that immunization with live attenuated vaccine not be used in pregnancy unless the risks from an existing epidemic or likely exposure to disease outweighs the risk of immunization.

Tularemia vaccine, by means of a live attenuated agent, is effective in preventing disease in people at high risk of exposure such as laboratory personnel working with *F. tularensis*.[2] However, the safety of this vaccine has not been established in pregnancy. In the aforementioned case study the course of pregnancy was reported in a patient inadvertently immunized with live attenuated tularemia vaccine near the time of fertilization.

It is recommended that a woman in the reproductive age group be counseled as to the possibly hazardous nature of a live attenuated vaccine so that she may elect to avoid conception during a period of potential fetal hazard.

The opinions and assertions contained herein are those of the authors and not to be construed as official or as reflecting the views of the Navy Department, the Naval Service at large, or the Department of Defense.

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Endometrial changes after administration of gossypol for menorrhagia

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GOSSYPOL is a yellowish phenolic compound isolated from the seed, stem, or root of the cotton plant. Chinese scientific workers discovered in the 1950s through mass investigation that cooking with crude cottonseed oil could lead to infertility.[1] Menostasis and atrophy of the uterus have also been seen among patients after treatment with gossypol.

Gossypol acetic acid was administered orally to 67 patients (36 to 50 years old) clinically diagnosed as having menorrhagia, leiomyoma, and endometriosis. The therapeutic dose is 20 mg/day for 2 months and the maintenance dose, 25 mg two times a week for 3 months. Endometrial specimens were taken from various patients at different intervals after the treatment to determine the histologic changes of the endometrium. In 47 of the 67 cases studied, endometrial specimens were taken both before and after the treatment.

Gossypol was administered orally for 1 to 3 months in 26 cases, for 4 months in 12 cases, for 5 months in 19 cases, for 6 months in 6 cases, for 7 months in 1 case, for 10 months in 1 case, and for 1 year in 1 case; in 1 case the duration of treatment was not clear.

Atrophy of the endometrium is the main morphologic change seen following the treatment (Figs. 1 and 2). Since the endometrium is usually extremely thin, curettage specimens are not easily obtained. Sometimes only a very small amount of thin, grayish membranous tissue is obtained.

The changes observed are as follows:

1. The superficial epithelium of the endometrium is

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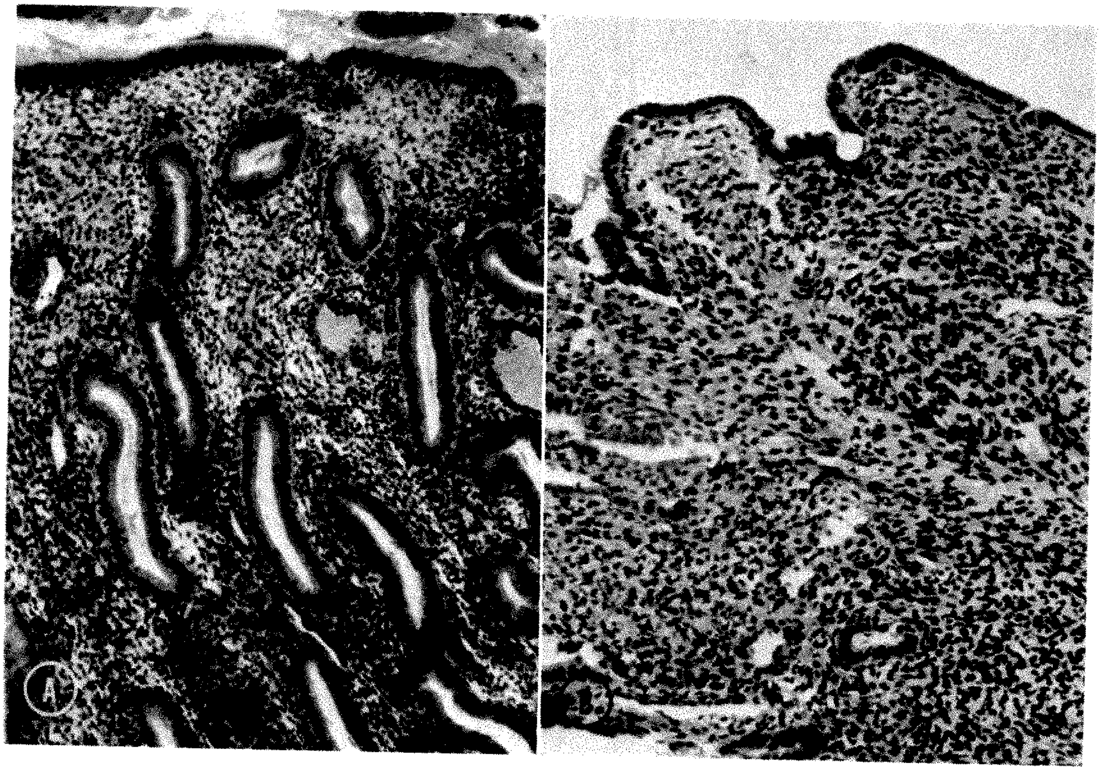

Fig. 1. *A:* Early secretory endometrium before treatment in 36-year-old woman. (Hematoxylin and eosin. ×125.) *B:* Atrophic endometrium after 6 months of treatment. (Hematoxylin and eosin. ×250.)

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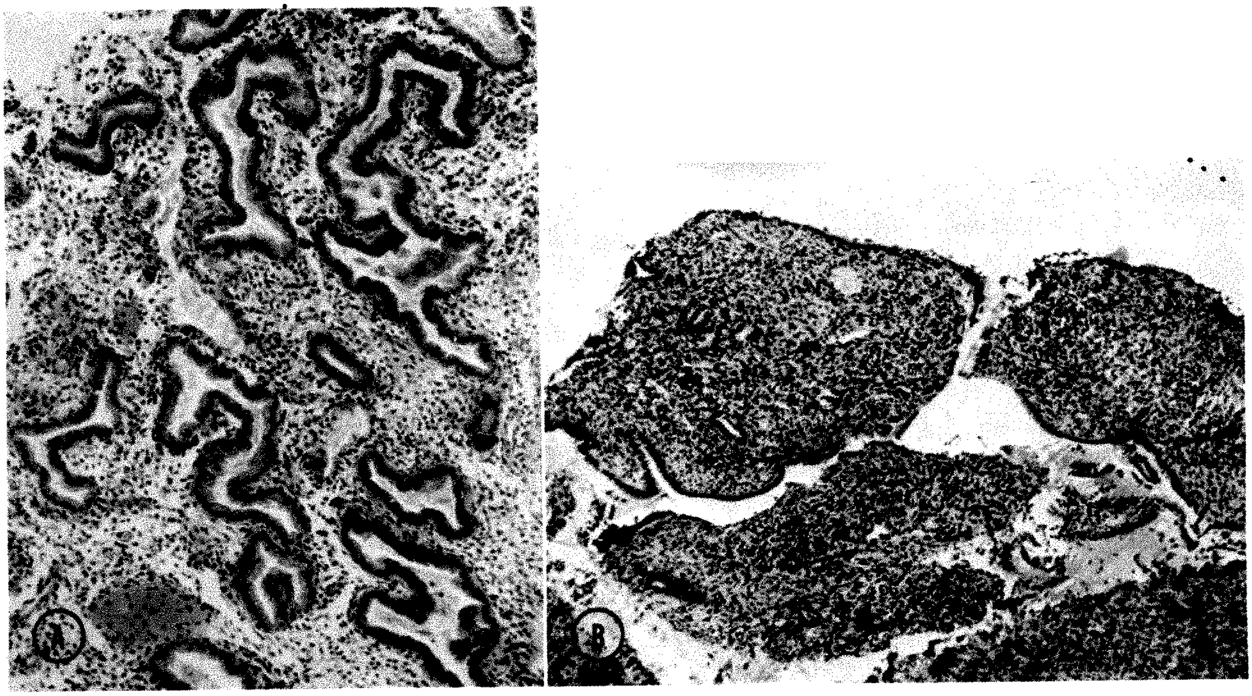

Fig. 2. *A:* Midsecretory endometrium before treatment in 46-year-old woman. (Hematoxylin and eosin. ×125.) *B:* Atrophic endometrium after 6 months of treatment. (Hematoxylin and eosin. ×125.)

Table I. Histologic changes in endometrium following oral administration of Gossypol

Time in months	*Histologic features*			
	Proliferative phase	*Secretory phase*	*Cystic hyperplasia*	*Atrophy*
1 to 3	7	7	8	4
4	2	2	2	6
5	2	—	1	16
6	3	—	—	3
7	—	—	—	1
10	—	—	—	1
12	—	—	—	1
Unknown	—	—	1	—
Total	14	9	12	32

Table II. Relation of the duration of treatment to atrophy of the endometrium

Time in months	*No. of cases*	*No. of cases with atrophy*
1 to 3	26	4
4	12	6
5	19	16
6	6	3
7	1	1
10	1	1
12	1	1
Unknown	1	—
Total	67	32

composed of a single layer of cells which is flattened, cubical, or low columnar in shape.

2. The number of glands is reduced and the stroma is scanty.

3. Glands, small, round, and uniform in shape, are widely scattered. They are lined by a single layer of epithelium, the lining cells of which are cubical or low columnar in shape. Neither transparent cells of large or small types nor ciliated cells are found in the glandular epithelium, which is an important indication of estrogenic activity.[2, 3] The stroma cells of the endometrium look like fibrous tissue cells.

Before gossypol is given, the endometrium appears to be in the secretory phase or the proliferative phase or demonstrates cystic hyperplasia. After gossypol administration, 32 cases showed varying degrees of atrophy, whereas no atrophy took place in the other 35 cases. Only four of 26 cases in which the patients had been treated with gossypol for 1 to 3 months showed atrophy. Among these four cases, only one appeared atrophic after 2 months of treatment, while in the other three atrophy appeared after 3 months of treatment. Atrophy occurred in 16 of 19 cases after 5 months of treatment. All patients who received treatment for more than 6 months showed atrophic changes in the endometrium. Thus, it is evident that atrophy of the endometrium is closely related to the duration of treatment. The number of cases showing atrophy increases with the elongation of treatment (Tables I and II). Once the treatment is withdrawn, the endometrium gradually recovers, although it takes a period of about 6 months to reach full recovery.

It has been found that cooking with crude cottonseed oil can lead to scanty menstruation and menostasis. In one case (not one of the 67 cases mentioned above) menostasis actually occurred in a 32-year-old woman who had used crude cottonseed oil in cooking for 2 years. When she ceased using such oil in cooking, the endometrium gradually recovered, followed by the return of menstruation.

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The Trendelenburg position for obstetric ultrasound

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IN OBSTETRIC ULTRASOUND, the region of the lower uterine segment and the cervix is the most difficult to evaluate. Saunders[1] has demonstrated that the cervix is not always centrally located beneath the uterovesical fold. Hence, an accurate delineation of the interrelationships among the cervix, placenta, and fetus is often difficult or impossible to appreciate. A fluid-filled pessary has been suggested as one way to identify the position of the cervix ultrasonically.[1] This method, however, cannot be used in patients with suspected placenta previa since any pelvic manipulation may elicit bleeding. Occasionally, the endocervical canal can be visualized. Unfortunately, this landmark is not consistently demonstrated.

If the obstetrician is trained in ultrasound, then his interpretation of a particularly difficult scan may be improved by performing a pelvic examination independent of or simultaneously with real-time scanning.[2] Again, the possibility of placenta previa limits the applicability of this technique. The radiologist, however, must perform obstetric ultrasonic examinations without the benefit of pelvic manipulation. Hence, gentle movement of the fetus, changes in maternal position, and emptying and filling of the maternal bladder are the only means that he may apply to portray the various intrapelvic relationships to their best advantage. For example, the biparietal diameter is frequently difficult to obtain in breech presentation. By placing the patient in a lateral position, the relationship of the fetal vertex to the maternal abdomen may be altered sufficiently to permit an accurate biparietal measurement.

In order to better delineate the lower uterine segment, we suggest that the Trendelenburg position be used during obstetric ultrasonic examinations in the second trimester and early third trimester. This maternal position would be advantageous in four situations. First, cervical dilatation that might be obscured by a closely approximating fetal part can be better visualized. In an ultrasonic examination performed to

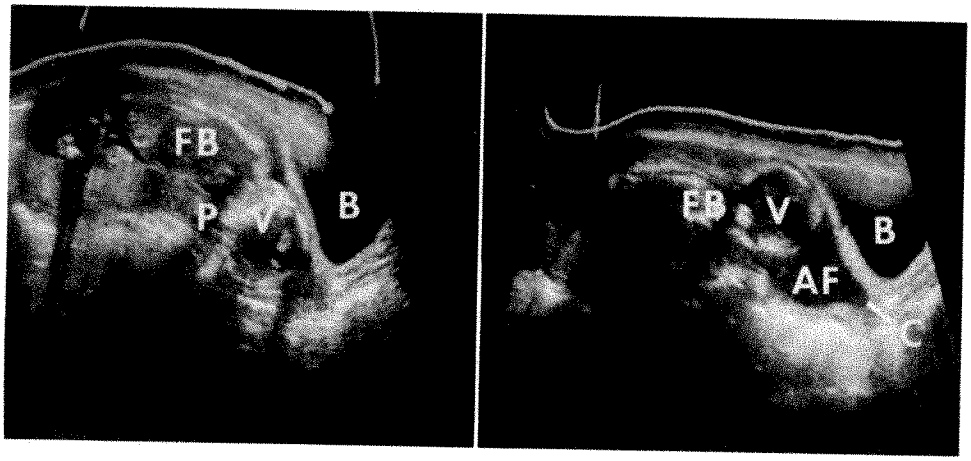

Fig. 1. Longitudinal scans. *Left,* Supine. *B* = bladder; *V* = vertex; *FB* = fetal body; and *P* = edge of placenta. *Right,* Trendelenburg position. Fetal vertex *(V)* has fallen away from region of cervix *(C),* and a dilating wedge of amniotic fluid *(AF)* is easily visualized. *B* = bladder; *FB* = fetal body.

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determine the gestational age and the possibility of incompetent cervix, careful longitudinal scans at 0.5 cm intervals failed to reveal any obvious cervical abnormalities (Fig. 1, *left*). Once the patient was placed in the Trendelenburg position, the fetus moved away from the lower uterine segment, and cervical incompetence could be readily demonstrated (Fig. 1, *right*). Second, sufficient elevation of the fetal vertex out of the pelvis with the mother in the Trendelenburg position may permit an accurate biparietal diameter determination when one was not possible with the patient in the routine supine position. Third, a low-lying posterior placenta can be evaluated without the acoustic shadowing of the presenting part (Fig. 2). Fourth, cervical position may be better delineated. While the importance of a full bladder to the ultrasound examination has been repeatedly emphasized, its influence on cervical position and size has not been fully appreciated. As the bladder fills, the cervix is passively pulled cephalad. If the bladder is too full, compression and elongation of the cervical canal occur, and the position of the canal will be altered.

Ultrasonic scanning with a full bladder and after voiding in both the horizontal and Trendelenburg positions assures an accurate assessment of the lower uterine segment, its contents, and the area of the cervix.

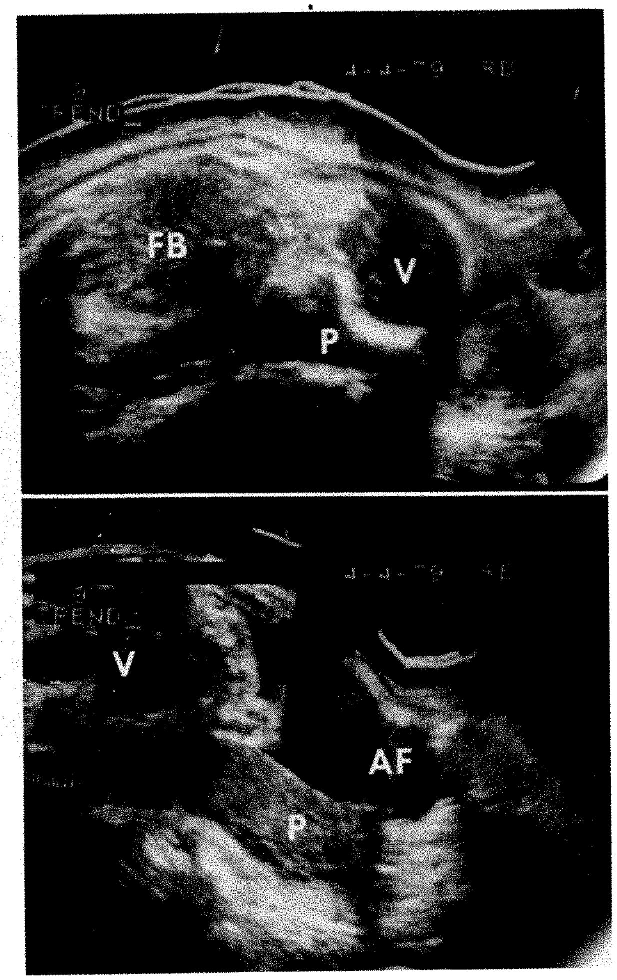

Fig. 2. Longitudinal scans. *Upper,* Supine. Fetal vertex *(V)* impairs visualization of the placenta *(P)*. *FB* = fetal body. *Lower,* Trendelenburg position. Posterior placenta *(P)* may now be visualized without the shadowing of a fetal part. *AF* = amniotic fluid; *V* = vertex.

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CORRESPONDENCE

Definition of sinusoidal fetal heart rate

To the Editors:

In their article, "Sinusoidal fetal heart rate. I. Clinical significance" (Am. J. Obstet. Gynecol. 136:587, 1980), Drs. Young, Katz, and Wilson reported their experience with 16 cases of so-called sinusoidal fetal heart rate (SHR). There were no fetal deaths in that series. Only one fetus (postdates with meconium) had a 5-minute Apgar score less than 7; pH determinations from the fetal scalp blood showed fetal acidosis (pH ≤7.20) in only four cases, three of which had intrapartum complications (postdates, meconium, partial abruptio placentae, and toxemia). This extraordinary favorable outcome contrasts with a perinatal mortality rate of 50% to 75% quoted by the authors from the literature. SHR patterns are very rare occurrences with probably no more than 100 cases reported in the world literature to this date. However, the authors reported 16 cases among a study population of 4,800 patients (0.3%).

The discrepancy in incidence and outcome is probably best explained by a liberal definition of SHR employed by the authors: SHR is a "FHR pattern of regular variability resembling a sine wave, with a relatively fixed periodicity of 3 to 5 cpm, and an amplitude of 5 to 40 bpm from the baseline FHR, lasting at least 10 minutes." With this definition we now find 24 episodes (1.2%) of so-called SHR among 1,996 intrapartum FHR tracings which we recently studied.[1] All of these were transient, lasting 10 to 45 minutes. There was no associated fetal mortality. Two fetuses had 5-minute Apgar scores less than 7. Both of these exhibited other FHR abnormalities besides the sinuslike FHR changes. The fetal outcome in each group of SHR defined by amplitude and frequency of oscillations was not different from that observed in retrospective groups of variability patterns defined by the same criteria (Table I).

The fetal prognosis does not appear to depend on the so-called SHR but on complications during labor and delivery and certain associated FHR patterns which by themselves are significantly associated with poor fetal outcome. It is our impression that the authors have described mostly variants of FHR variability patterns.

Table I. FHR variability and sinuslike FHR changes (so-called sinusoidal FHR) defined by oscillatory amplitude and frequency: Association with 5-minute Apgar scores <7

	FHR variability			*Sinuslike FHR*		
		Apgar score <7			*Apgar score <7*	
	N	*n*	*% of N*	*N*	*n*	*% of N*
Amplitude:						
0-5 bpm						
All	551	50	9.1	0	0	0.0
Uncomplicated	36	1	2.8	0	0	0.0
Complicated	515	49	9.5	0	0	0.0
6-25 bpm						
All	1,307	27	2.1	16	1	6.3
Uncomplicated	420	5	1.2	5	0	0.0
Complicated	887	22	2.5	11	1	9.1
>25 bpm						
All	114	7	6.1	8	1	12.5
Uncomplicated	22	0	0.0	2	0	0.0
Complicated	92	7	7.6	6	1	16.6
Total	1,972	84	4.3	24	2	8.3
Frequency:						
<3 opm						
All	13	8	61.5	3	1	33.3
Uncomplicated	0	0	0.0	0	0	0.0
Complicated	13	8	61.5	3	1	33.3
3-6 opm						
All	73	13	17.8	4	0	0.0
Uncomplicated	26	1	3.8	1	0	0.0
Complicated	47	12	25.5	3	0	0.0
>6 opm						
All	1,886	63	3.3	17	0	0.0
Uncomplicated	421	5	1.2	4	0	0.0
Complicated	1,465	58	4.0	13	1	7.6
Total	1,972	84	4.3	24	2	8.3

bpm = Beats per minute; opm = oscillations per minute.

The FHR tracing of the authors shown in Fig. 3 may be characterized as a tracing with normal FHR variability (amplitude of oscillations 10 bpm, frequency of oscillations 10 opm), mild variable decelerations, and absence of accelerations over a period of approximately 10 minutes of monitoring. The fetal prognosis should be excellent, particularly if accelerations were demonstrable earlier or could be elicited by manipulations of the fetus.

The fetal monitoring record in Fig. 4 shows a FHR with marked variability (oscillatory amplitude 40 bpm, oscillatory frequency approximately 4 opm). The pattern appears to be limited in its apparent regularity to approximately 4 minutes as indicated by smaller FHR undulations in the beginning and at the end of the displayed tracing. In our experience a "saltatory" oscillatory amplitude (>25 bpm) is likely to worsen fetal prognosis only when associated with some other abnormality in labor.[1]

Clearly, criteria need to be established to separate prognostically insignificant variants of FHR variability from sinusoidal FHR changes associated with grave fetal outcome. From the study of sinuslike FHR changes displayed in seven pertinent articles (references will be sent upon request) we conclude that the intrapartum sinusoidal FHR is characterized by the following:

1. Abnormal FHR patterns such as sudden onset of bradycardia, late and variable decelerations precede the SHR.
2. The preceding abnormal FHR patterns and consequently the SHR is associated with obvious precipitating factors and events such as (partial) abruptio placentae, placental insufficiency in postdate pregnancy, uterine hyperstimulation, cord compression, and others.
3. SHR patterns are transient.
4. The amplitudes of the sinus waves increase gradually to a maximum and then decrease in a similar fashion.
5. The undulations are regular and of smooth contour. FHR irregularities or variability changes within the sinus waves are minimal or absent.
6. There is rare or no alteration in the pattern in association with uterine contractions.
7. Abnormal FHR patterns such as bradycardia and absence of FHR variability follow the SHR. Exceptions are rare.
8. The outcome of pregnancy is fetal death or a severely depressed infant.

In summary, we join other authors in concluding that the SHR is an agonal FHR likely reflecting hypoxia and deranged nervous control of the fetal heart. While the pattern may appear in a wide variety of oscillatory amplitudes and frequencies, the smoothness and regularity of the sinus waves are characteristic in almost all reported cases and are associated with poor fetal outcome. Intrapartum sinuslike FHR changes should not be regarded as sinusoidal but as (usually benign) variants of variability unless any of the ominous FHR patterns mentioned above precede or follow the SHR. Dr. Young and his associates have shown that sinuslike FHR changes may occur sporadically in normal fetuses. They may also be caused by drugs affecting the central nervous system such as alphaprodine.[2] We suggest that these benign patterns be called "sinuslike variability" or "sinuforme variability."

The prognosis of antepartum sinuslike FHR changes is less easily determined because preceding FHR changes are often not recorded. A simple test may be the interruption of a sinuslike FHR by mechanical stimulation. We noted the conversion of what appeared to be a sinusoidal FHR to normal FHR variability by stimulation of the fetus in two cases. A fetus with deranged nervous control of the fetal heart is not likely to respond in this manner.

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Reply to Dr. Krebs

To the Editors:

In response to Dr. Krebs' detailed letter I will begin by disputing his statement that our paper reports a "discrepancy in incidence" of SHR in the world literature, although, as we stated, our *outcome* is clearly different from that of previous reports. Our incidence was 0.3%, certainly a low rate, and Dr. Krebs offers no citation of the literature to substantiate his statement of an incidence less than this.

Dr. Krebs refers to the "liberal definition of SHR employed" to explain the suggested increase in incidence. In fact, while the phraseology of the definition is mine, it is based on the original definition of Manseau and associates[1] and the observations of Baskett and Koh[2] and Rochard and associates,[3] as clearly stated in the first paragraph of the paper, with the references cited there as well. Thus, the definition is from the literature, the same literature which originally reported the high perinatal mortality with SHR, as also mentioned in the first paragraph of the paper. Further, when Dr. Krebs used this definition retrospectively in a review of his own material from a previous unrelated study, his incidence was an "underwhelming" 1.2%, hardly evidence of a liberal definition.

Interestingly, his review of these data is supportive of our own report, although lacking biochemical studies and relying solely on Apgar scores. However, he has misinterpreted the data from his Table I when he states, "The fetal outcome in each group of SHR defined by amplitude and frequency of oscillations was not different from that observed in retrospective groups of variability patterns defined by the same criteria." Our paper did not ascribe any significance to frequency, and Dr. Krebs' table does not delineate a separate group of 3 to 5 cycles per minute for SHR, although these cases would be included in the groups of 6 opm and above. However, in the amplitude section of his table, the cases which Dr. Krebs calls "sinuslike FHR" show a much higher occurrence of Apgar scores under 7 in the range over 25 bpm and in the 6 to 25 bpm range. This is consistent with our report that there is a tendency to a falling pH and a worse perinatal outcome with increasing amplitude, with a breaking point about 15 bpm. The "sinuslike" patterns were associated with two to three times the incidence of low Apgar scores seen in the nonsinus groups. Thus, the fetal outcomes *were* different according to Dr. Krebs' data and interpretation of the FHR patterns based on our definition of SHR.

Dr. Krebs' interpretations of the figures showing minor and major SHR patterns are most likely based on his terminology, e.g., oscillations per minute. Our usage of cycles per minute refers to a cycle measure from peak to peak of a sine wave. Thus, the frequency in Fig. 3 is 5 cpm not 10 opm, and in Fig. 4, 2 to 3 cpm, not 4 opm. Similarly, amplitude refers to height from a baseline and may be positive or negative, but not the sum of the upward and downward deflections from the baseline. Fig. 4 shows approximately 5 minutes of a pattern which lasted much longer, with some variation in amplitude. As stated in the paper, patterns with a duration of less than 10 minutes were not considered.

Dr. Krebs' attempt to establish criteria for sinusoidal FHR is welcome. We have been engaged in the same pursuit. However, of the eight criteria he lists, based on his review of the literature, Numbers 1, 7, and 8 are of profound prognostic significance by themselves, even in the absence of a SHR. His inclusion of SHR as a prognostically poor pattern is tautologous. Numbers 2, 3, 4, and 5 often are seen with SHR patterns, but not always. Number 6 is correct. Unfortunately, these eight criteria fail to define the SHR pattern. They merely describe phenomena which may be associated with it. The definition of SHR derived from the literature is quite clear and allows diagnosis by visual or computer analysis of a FHR tracing. The SHR is a FHR pattern of regular variability resembling a sine wave, with a relatively fixed periodicity of 3 to 5 cpm and an amplitude of 5 to 40 bpm from the baseline FHR, lasting at least 10 minutes.[1–3] I am not familiar with any literature on Dr. Krebs' terms "sinuslike variability" and "sinuforme variability."

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β-Endorphin in obstetrics: Effects on fetus

To the Editors:

Oyama and co-workers recently published their exciting and encouraging results following β-endorphin administration to 14 parturient patients during labor and delivery.[1] Although intrathecal administration of β-endorphin or similar peptide analogues may well play a significant clinical role in the future in amelioration of intrapartum and postpartum pain, a word of caution is indicated. The authors referenced their statement that β-endorphin does not cross the blood brain barrier (BBB)[2] but excluded mention of the controversy that exists over this issue. In spite of its size, β-endorphin (and other sizable neuropeptides) may gain access to the central nervous system by crossing the BBB where there is less of an impediment to diffusion, i.e., the area postrema or the circumventricular organs. This issue and potential explanations are addressed in two recent publications.[3, 4] As a clinical correlate, Kline and associates administered intravenous β-endorphin to six psychiatric patients and noted behavioral improvements, thus implying that some β-endorphin might have reached receptors in the central nervous system.[5] This point in no way detracts from the authors' observations, but intrathecally administered medications do achieve measurable levels in the peripheral blood[6] to which the fetus is exposed. Likewise, stress does produce measurable increases in peripheral blood β-endorphin concentrations,[4] again with some (unmeasured to date) fetal exposure. Kastin and colleagues[3] have further reported that newborn rats treated during the first week of life with 50 μg of subcutaneously administered β-endorphin were in a state of analgesia when tested at 90 days of age.[3] Absence of analgesia in the infants exposed in the study of Oyama and associates would be reassuring, although it was not reported.

It is unlikely that the human fetus would be exposed significantly following the maternal intrathecal administration of 1 mg of β-endorphin since three physiologic barriers must be traversed (maternal and fetal BBB and placenta). However, the effects of the potency of such neuropeptides and their degradation products on the developing central nervous system of the fetus require a cautious approach. Also smaller doses of β-endorphin, as the authors suggest, may well suffice.

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Prostaglandin synthetase inhibitors should not be referred to as antiprostaglandin drugs

To the Editors:

I read with interest Dr. Ralph Sortor's Letter to the Editor on page 696 of the March 1, 1980, issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY entitled, "Antiprostaglandin drugs and Von Willibrand's disease."

I would like to express my disagreement with the use of the word "antiprostaglandin" referred to drugs such as aspirin, indomethacin, etc. These drugs should be correctly referred to as prostaglandin synthetase inhibitors. The word antiprostaglandin implies that these drugs interfere with the action of prostaglandin, which to my knowledge they do not. These drugs interfere with the endogenous synthesis of prostaglandin. If one administers aspirin and prostaglandin simultaneously, the aspirin will not interfere with the action of the prostaglandin. In fact, I have some preliminary data in animals that suggest that aspirin will enhance and prolong the fever induced by exogenously administered PGE_2. This might be expected since it has been shown by Hansen (Prostaglandins 8:95, 1974) that aspirin inhibits the action of 15-hydroxy prostaglandin dehydrogenase, the enzyme responsible for the initial step in the catabolism of PGE_2.

The JOURNAL's referral to aspirin as an "antiprostaglandin" drug would tend to encourage its use in the treatment of fever induced by PGE_2 vaginal suppositories. While I know of no evidence that aspirin in the human will enhance the fever induced by PGE_2 vaginal suppositories, I also know of no evidence that it will reduce it. Until data are presented that aspirin is beneficial in this regard, I would encourage obstetricians to avoid this drug and attempt to reduce PGE_2-induced fever by some other means.

In conclusion, I would like to request that in future communications the Editorial Staff of the JOURNAL refer to drugs such as aspirin and indomethacin as prostaglandin synthetase inhibitors and not as antiprostaglandin drugs.

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Reply to Dr. Nelson

To the Editors:

Thank you for your letter and the information given as to the correct nomenclature regarding prostaglandin synthetase inhibitors.

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Therapy of endometriosis and dysmenorrhea

To the Editors:

I would like to comment on Dr. Karnaky's letter, entitled "Oral contraception and endometriosis" (AM. J. OBSTET. GYNECOL. 135:279, 1979). I am not sure whether Dr. Karnaky has ever proved that his good results are secondary to the addition of vitamins or are due to the basic stilbene. Except in women who are symptomatic in the menopause and climacteric, I have not used stilbestrol or any of the stilbenes since Ferguson presented his data on pregnancy salvage in the early fifties in this JOURNAL.

Having been involved in the experimental development and use of several of the oral contraceptives and progestins, I have no hesitancy in using these for the control of dysmenorrhea, providing the patient is mature and fully developed. In others I now use the antiprostaglandins. My experience is similar to that of Dr. Karnaky in only one way—my failures occur with adenomyosis, not extending endometriosis. I still used pseudopregnancy with the contraceptives in proved endometriosis, and my failures have been few. I do not have a research group to evaluate my data, but I am fully aware of my successes and the few failures.

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Reply to Dr. Eichner

To the Editors:

The statement by Dr. Eichner that he does not use stilbestrol in women because it has caused clear cell adenocarcinoma in girls (pregnant and nonpregnant women may be different?) takes the cake.

I use the example of yellow teeth from tetracycline administration during pregnancy. If he actually used that common-sense statement as a guideline, then he would not use many drugs. This is similar to a gynecologist's writing, "I stopped doing orchiectomy in women."

Only stilbestrol-hexestrol blocks these active estrogenic sites so that endometriosis undergoes atrophy. No other medications have been shown to do this.

I broke with the drug companies after working with them for 30 years when they tried to get me to say that progestins were superior to stilbestrol and hexestrol in menstrual disorders. I was seeing over 200 such patients twice monthly and the progestins were producing inferior or no results compared to stilbestrol and hexestrol when being tested side by side. The amount of estrogens in these progestins is far too small to produce any results. We found progestins useful only for contraception. They should be discontinued in all gynecologic conditions.

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Errata

In the article by Sehgal and Hitt, entitled "Plasma volume expansion in the treatment of pre-eclampsia," in the September 15, 1980, issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, in the eleventh line of the first column on page 166, the sentence should have read: "MABP was calculated by

$$\frac{\text{systolic} + 2\ \text{diastolic"}}{3}.$$

In the article by Porreco, Merritt, and Gluck, entitled "Effect of prolactin on phospholipid biosynthesis by alveolar cell carcinoma (A-549) in monolayer tissue culture," in the April 15, 1980, issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, on page 1071, a supporting grant was omitted. This footnote should have read: "Supported by National Institutes of Health Grant HD 10622.'

BOOKS

Books received

Breastfeeding Handbook. Johanna Goldfarb and Edith Tibbetts. 256 pages, illustrated. Hillside, New Jersey, 1980, Enslow Publishers. $14.95 (soft cover).

Cisplatin. Current Status and New Developments. Edited by Archie W. Prestayko, Stanley T. Crooke, and Stephen K. Carter. 527 pages, illustrated. New York, 1980, Academic Press, Inc. $30.00.

Concepts in Cancer Care. A Practical Explanation of Radiotherapy and Chemotherapy for Primary Care Physicians. Jay Scott Cooper and Donald J. Pizzarello. 273 pages, illustrated. Philadelphia, 1980, Lea & Febiger. $16.50.

Diagnostic Ultrasound. Physical Principles and Exercises. Frederick W. Kremkau. 211 pages, illustrated. New York, 1980, Grune & Stratton, Inc. $19.50.

Human Labor & Birth. Ed. 4. Harry Oxorn. 726 pages, illustrated. New York, 1980, Appleton-Century-Crofts. $17.95 (soft cover).

Microsurgery in Infertility. Alain Audebert, Michel Cognat, Ettore Cittadini, and Robert Schoysman. 162 pages, illustrated. Palermo, Italy, 1980, Cofese. No price listed.

Obstetric Analgesia and Anesthesia. Edited by Gertie F. Marx and Gerard M. Bassell. 415 pages, illustrated. Amsterdam, 1980, Excerpta Medica. $75.50.

Obstetrics and Gynecology Annual. Vol. 9. Edited by Ralph M. Wynn. 390 pages, illustrated. New York, 1980, Appleton-Century-Crofts. $29.50.

Perinatal Medicine. Clinical and Biochemical Aspects. Volume 1. Edited by Manohar Rathi and Sudhir Kumar. New York, 1980, Hemisphere Publishing Corporation. $35.00.

The Rape Crisis Intervention Handbook. A Guide for Victim Care. Edited by Sharon L. McCombie. 235 pages. New York, 1980, Plenum Press. $19.50.

Recent Advances in Obstetrics and Gynaecology. Number 13. Edited by John Stallworthy and Gordon Bourne. 300 pages, illustrated. New York, 1979, Churchill Livingstone. No price listed.

Recent Advances in Ultrasound Diagnosis. 2. Proceedings of the International Symposium on Recent Advances in Ultrasound Diagnosis, Dubrovnik, October 1-5, 1979. Edited by Asim Kurjak. 560 pages, illustrated. Amsterdam, 1980, Excerpta Medica. $97.50.

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Volume 138
September-December, 1980
The C. V. Mosby Company
St. Louis
1980

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Printed in the United States of America

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OBSTETRICS AND GYNECOLOGY

SALARIES highly competitive and negotiable. Instruction is in English. Interviews scheduled during late-1980 and early-1981.

CONTRACTS are for one year and renewable. Clinicians hold faculty appointments.

BENEFITS include free furnished housing, airtickets to and from Saudi Arabia one time each year for a family of four, 60-day vacation with pay, generous overweight and educational allowances. No Saudi income tax.

Please send curriculum vitae with current telephone numbers, and the names and addresses of three references to:

Dr. Tawfiq Tamimi, Dean
College of Medicine, King Faisal University

c/o Saudi Arabian
Educational Mission
2425 West Loop South
Houston, TX 77027

OR

P.O. Box 2
Sunbury-on-Thames
Middlesex TW16 5JP
England

Flagyl® (metronidazole) 250 mg.

Warning

Metronidazole has been shown to be carcinogenic in mice and possibly carcinogenic in rats. (See *Warnings*.) Unnecessary use of the drug should be avoided. Its use should be reserved for the conditions described in the *Indications* section.

Indications: For the treatment of symptomatic trichomoniasis in females and males when the presence of the trichomonad has been confirmed by appropriate laboratory procedures (wet smears and/or cultures). Also for the treatment of asymptomatic females when the organism is associated with endocervicitis, cervicitis, or cervical erosion. *T. vaginalis* infection is a venereal disease and therefore, asymptomatic sexual partners of treated patients should be treated simultaneously if the organism is present. The decision to treat an asymptomatic male partner who has a negative culture, or one in whom no culture has been attempted, is an individual one. In any event, the consort should be treated with Flagyl in cases of reinfection. Flagyl is also indicated for acute intestinal amebiasis (amebic dysentery) and for amebic liver abscess.

Contraindications: Evidence or history of blood dyscrasia, active organic disease of the CNS, the first trimester of pregnancy and a hypersensitivity to metronidazole.

Warnings: Flagyl should not be used in the first trimester of pregnancy (see *Contraindications*). Use with discretion during the second and third trimesters of pregnancy and restrict to those pregnant patients not cured by topical measures. Flagyl (metronidazole) is secreted in the breast milk of nursing mothers. It is not known whether this can be injurious to the newborn. If used during lactation, an alternative method of feeding may be elected.

Tumorigenicity Studies in Rodents. Metronidazole has shown evidence of tumorigenic activity in a number of studies involving chronic, oral administration in mice and rats.

Most prominent among the effects in the mouse was the promotion of pulmonary tumorigenesis. This has been observed in all five reported studies in that species, including one study in which the animals were dosed on an intermittent schedule (administration during every fourth week only). One of the mouse studies revealed increases in the incidence of malignant lymphomas as well as pulmonary neoplasms associated with lifetime feeding of the drug. All these effects are statistically significant.

In two long-term toxicity studies in the rat there was a statistically significant increase in the incidence of various neoplasms, particularly mammary tumors, among female rats on metronidazole over that noted in the control groups.

Two lifetime tumorigenicity studies in hamsters have been reported to be negative.

Metronidazole has been reported to potentiate the anticoagulant effect of coumarin and warfarin resulting in a prolongation of prothrombin time. This possible drug interaction should be considered when Flagyl is prescribed for patients on this type of anticoagulant therapy.

Precautions: Mild leukopenia has been reported during Flagyl (metronidazole) use: total and differential leukocyte counts are recommended before and after treatment with the drug, especially if a second course is necessary. Flagyl may interfere with certain chemical analyses for SGOT. Avoid alcoholic beverages during Flagyl therapy because abdominal cramps, nausea, vomiting, headaches and flushing may occur. Discontinue Flagyl promptly if abnormal neurologic signs occur. Exacerbation of candidiasis may occur. In amebic liver abscess, aspirate pus during metronidazole therapy.

Adverse Reactions: Nausea, headache, anorexia, vomiting, diarrhea, epigastric distress, abdominal cramping, constipation, a metallic, sharp and unpleasant taste, furry tongue, glossitis and stomatitis possibly associated with a sudden overgrowth of *Candida*, exacerbation of vaginal candidiasis, an occasional reversible moderate leukopenia, dizziness, vertigo, incoordination, ataxia, convulsive seizures, peripheral neuropathy, fleeting joint pains, confusion, irritability, depression, weakness, insomnia, mild erythematous eruptions, urticaria, flushing, nasal congestion, dryness of the mouth, vagina or vulva, pruritus, dysuria, cystitis, a sense of pelvic pressure, dyspareunia, fever, polyuria, incontinence, decrease of libido, proctitis, pyuria and darkened urine have occurred in patients receiving the drug. Patients receiving Flagyl may experience abdominal distress, nausea, vomiting, flushing or headache if alcoholic beverages are consumed. The taste of alcoholic beverages may also be modified. Flattening of the T-wave may be seen in ECG tracings.

SEARLE **Searle & Co.**
San Juan, Puerto Rico 00936

Address medical inquiries to:
G. D. Searle & Co.
Medical Communications Department, Box 5110
Chicago, Illinois 60680

941-R2

His trichomoniasis can ake her therapy useless unless they both take..

When the male is not treated for *Trichomonas vaginalis,* the female—no matter how well she cooperates with therapy—probably will become reinfected. To arrest this infection/reinfection cycle, both infected partners must be treated simultaneously.

The only systemic trichomonacide—In the female, trichomoniasis is a multiorgan disease. Trichomonads invade the vagina, and are often deeply entrenched in the endocervix, Skene's and Bartholin's glands, the urethra, and the bladder. The male genitourinary tract has seven possible sites of infection. Only the systemic action of Flagyl can eliminate sequestered trichomonads from these sites; topical treatments are ineffective.

Effective in both partners—Because it reaches infection sites via the bloodstream, Flagyl is the only trichomonacide that is effective in both men and women. And Flagyl is the only available agent of its kind.

The only effective trichomonacide for couples

Flagyl® 250-mg. tablets (metronidazole)

Please see adjacent page for brief summary of prescribing information.

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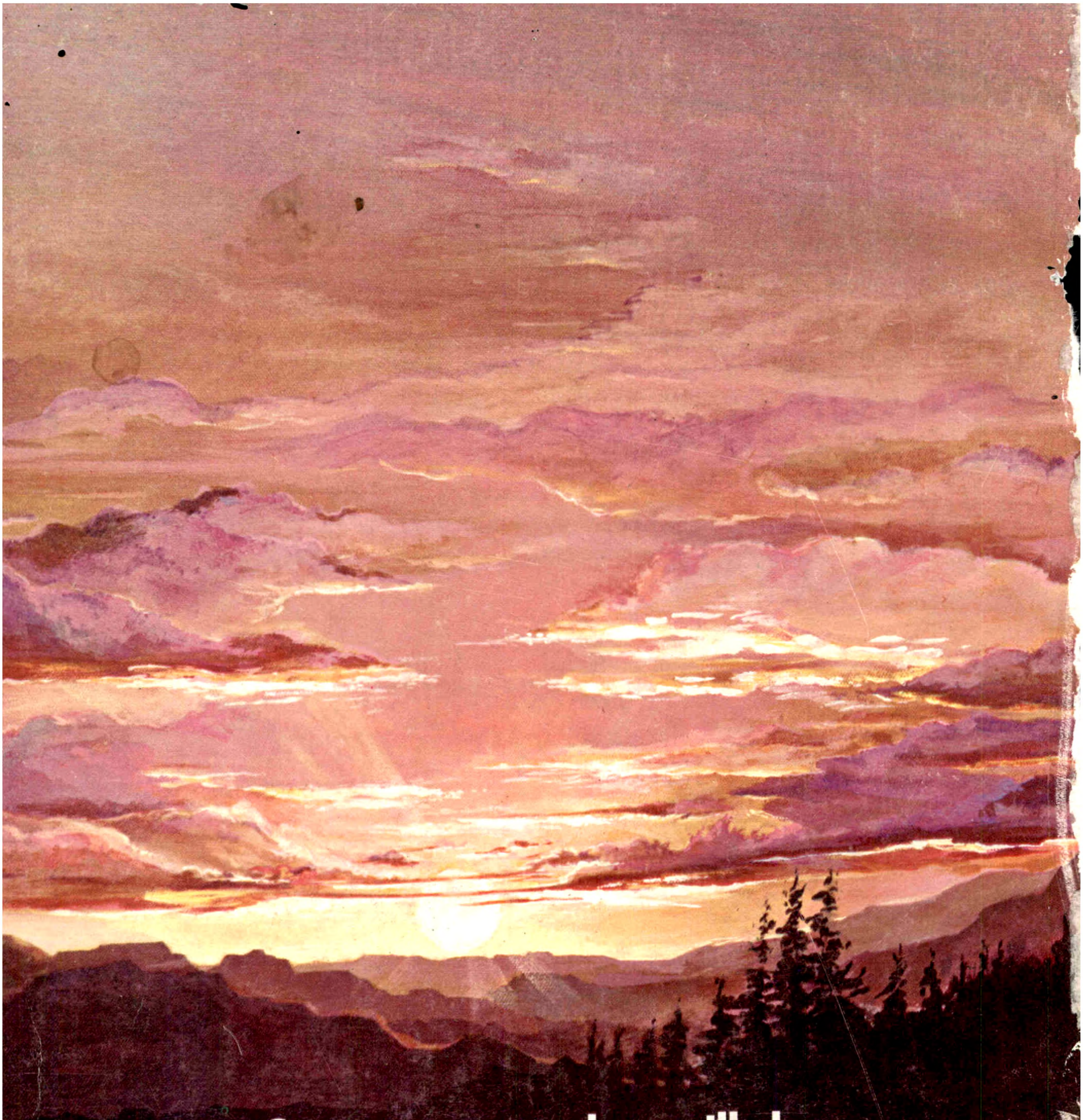

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